

## INHIBITORS OF PAPILLOMA VIRUS

### RELATED APPLICATIONS

Benefit of U.S. Provisional Application Serial No. 60/256,706, filed on  
5 December 18, 2000 is hereby claimed and said Application is incorporated  
herein by reference.

### FIELD OF THE INVENTION

The present invention relates to compounds, compositions and methods for  
the treatment of papilloma virus (PV) infection, particularly human papilloma  
10 virus (HPV). In particular, the present invention provides novel indane  
derivatives, pharmaceutical compositions containing such derivatives and  
methods for using these compounds in the treatment of papilloma virus  
infection. More particularly, the present invention provides compounds,  
compositions and methods for inhibiting papilloma virus DNA replication by  
15 interfering with the E1-E2-DNA complex during initiation of DNA replication.

### BACKGROUND OF THE INVENTION

Papillomaviruses are non-enveloped DNA viruses that induce  
hyperproliferative lesions of the epithelia. The papillomaviruses are  
20 widespread in nature and have been identified in higher vertebrates. Viruses  
have been characterized, amongst others, from humans, cattle, rabbits,  
horses, and dogs. The first papillomavirus was described in 1933 as cottontail  
rabbit papillomavirus (CRPV). Since then, the cottontail rabbit as well as  
bovine papillomavirus type 1 (BPV-1) have served as experimental prototypes  
25 for studies on papillomaviruses. Most animal papillomaviruses are associated  
with purely epithelial proliferative lesions, and most lesions in animals are  
cutaneous. In the human there are more than 75 types of papillomavirus that  
have been identified and they have been catalogued by site of infection:  
cutaneous epithelium and mucosal epithelium (oral and genital mucosa). The  
30 cutaneous-related diseases include flat warts, plantar warts, etc. The  
mucosal-related diseases include laryngeal papillomas and anogenital  
diseases comprising cervical carcinomas (Fields, 1996, Virology, 3rd ed.  
Lippincott – Raven Pub., Philadelphia, N.Y.).

There are more than 25 HPV types that are implicated in anogenital diseases, these are grouped into "low risk" and "high risk" types. The low risk types include HPV type 6, type 11 and type 13 and induce mostly benign lesions such as condyloma acuminata (genital warts) and low grade squamous intraepithelial lesions (SIL). In the United States there are 5 million people with genital warts of which 90% is attributed to HPV-6 and HPV-11. About 90% of SIL is also caused by low risk types 6 and 11. The other 10% of SIL is caused by high risk HPVs.

The high risk types are associated with high grade SIL and cervical cancer and include most frequently HPV types 16, 18, 31, 33, 35, 45, 52, and 58. The progression from low-grade SIL to high-grade SIL is much more frequent for lesions that contain high risk HPV-16 and 18 as compared to those that contain low risk HPV types. In addition, only four HPV types are detected frequently in cervical cancer (types 16, 18, 31 and 45). About 500,000 new cases of invasive cancer of the cervix are diagnosed annually worldwide (Fields, 1996, supra).

Treatments for genital warts include physical removal such as cryotherapy, CO<sub>2</sub> laser, electrosurgery, or surgical excision. Cytotoxic agents may also be used such as trichloroacetic acid (TCA), podophyllin or podofilox. Immunotherapy is also available such as Interferon or Imiquimod. These treatments are not completely effective in eliminating all viral particles and there is either a high cost incurred or uncomfortable side effects related thereto. In fact, there are currently no effective antiviral treatments for HPV infection since recurrent warts are common with all current therapies (Beutner & Ferenczy, 1997, Amer. J. Med., 102(5A), 28-37).

The ineffectiveness of the current methods to treat HPV infections has demonstrated the need to identify new means to control or eliminate such infections. In recent years, efforts have been directed towards finding antiviral compounds, and especially compounds capable of interfering with viral replication at the onset of infection (Hughes, 1993, Nucleic Acids Res.

21:5817-5823).

The life cycle of PV is closely coupled to keratinocyte differentiation. Infection is believed to occur at a site of tissue disruption in the basal epithelium.

5 Unlike normal cells, the cellular DNA replication machinery is maintained as the cell undergoes vertical differentiation. As the infected cells undergo progressive differentiation the viral genome copy number and viral gene expression in turn increase, with the eventual late gene expression and virion assembly in terminally differentiated keratinocytes and the release of viral

10 particles (Fields, *supra*).

The coding strands for each of the papillomavirus contain approximately ten designated translational open reading frames (ORFs) that have been classified as either early ORFs or late ORFs based on their location in the

15 genome. E1 to E8 are expressed early in the viral replication cycle, and two late genes (L1 and L2) encode the major and minor capsid proteins respectively. The E1 and E2 gene products function in viral DNA replication, whereas E5, E6 and E7 are expressed in connection with host cell proliferation. The L1 and L2 gene products are involved in virion structure.

20 The function of the E3, E4 and E8 gene products is uncertain at present.

Studies of HPV have shown that proteins E1 and E2 are both essential and sufficient for viral DNA replication *in vitro* (Kuo et al., 1994, J. Biol. Chem. 30: 24058-24065). This requirement is similar to that of bovine papillomavirus

25 type 1 (BPV-1). Indeed, there is a high degree of similarity between E1 and E2 proteins and the ori-sequences of all papillomaviruses (PV) regardless of the viral species and type (Kuo et al., 1994, *supra*).

Evidence emanating from studies of BPV-1 have shown that E1 possesses

30 ATPase and helicase activities that are required in the initiation of viral DNA replication (Seo et al., 1993a, Proc. Natl. Acad. Sci. USA 90:702-706; Yang et al., 1993, Proc. Natl. Acad. Sci. 90:5086-5090; and MacPherson et al., 1994, 204:403-408).

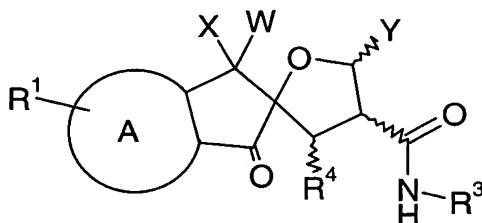
The E2 protein is a transcriptional activator that binds to E1 protein and forms a complex that binds specifically to the ori sequence (Mohr et al., **1990**, Science 250:1694-1699). It is believed that E2 enhances binding of E1 to the BPV origin of replication (Seo et al., **1993b**, Proc. Natl. Acad. Sci., 90:2865-2869). In HPV, Lui et al. suggested that E2 stabilizes E1 binding to the ori (**1995**, J. Biol. Chem., 270(45):27283-27291).

To thwart this disease, a chemical entity that would interfere with viral DNA replication is therefore desirable. The present invention therefore provides such compounds, compositions or methods that inhibit papilloma viral replication. More particularly, the compounds and composition of the present invention interfere with the E1-E2-DNA complex during the viral replication cycle.

The present description refers to a number of documents, the content of which is herein incorporated by reference.

## SUMMARY OF THE INVENTION

In a first aspect, the invention provides a compound of formula (I), or its enantiomers or diastereoisomers thereof:



(I) (form 1)

wherein:

**A** is a 5- or 6-membered homocyclic ring, or a 5- or 6-membered heterocyclic ring containing 1 or more heteroatoms selected from N, O and S;

**X** is H and **W** is OH; or **X** and **W** together form a carbonyl group or an epoxide;

**R**<sup>1</sup> is H; or one or two substituents independently selected from the group consisting of: hydroxy; halo; lower alkyl; lower alkoxy; lower thioalkyl; haloalkyl (e.g. trifluoromethyl); or  $-C(O)R^2$  wherein **R**<sup>2</sup> is lower alkyl, aryloxy or benzyloxy;

**Y** is phenyl optionally mono- or di-substituted with **R**<sup>5</sup> or  $C(O)R^6$ , wherein **R**<sup>5</sup> is lower alkyl, lower cycloalkyl, lower alkoxy, halo, hydroxy, nitrile or trifluoromethyl, and **R**<sup>6</sup> is lower alkyl, lower cycloalkyl, lower alkoxy, hydroxy or trifluoromethyl; said phenyl ring being optionally fused with a saturated or unsaturated 4 to 6-membered ring optionally containing a heteroatom selected from N, O and S;

or **Y** is a heterocycle (Het) containing one or more heteroatom selected from N, O or S, said Het optionally mono- or di-substituted with **R**<sup>5</sup> or  $C(O)R^6$ , wherein **R**<sup>5</sup> and **R**<sup>6</sup> are as defined above; said Het being optionally fused with a saturated or unsaturated 4 to 6-membered ring optionally containing a heteroatom selected from N, O and S;

or **Y** is ethylene-phenyl, said ethylene moiety being optionally mono-substituted with lower alkyl, wherein said phenyl ring is optionally mono- or di-substituted with **R**<sup>5</sup> or  $C(O)R^6$ , wherein **R**<sup>5</sup> and **R**<sup>6</sup> are as defined above; said phenyl ring being optionally fused with a saturated or unsaturated 4- to 6-

membered ring optionally containing a heteroatom selected from N, O and S;

or Y is ethylene-Het, said ethylene moiety being optionally mono-substituted with lower alkyl, wherein Het is optionally mono- or di-substituted with  $R^5$  or  $C(O)R^6$ , wherein  $R^5$  and  $R^6$  are as defined above; said Het being optionally

- 5 fused with a saturated or unsaturated 4 to 6-membered ring optionally containing a heteroatom selected from N, O and S;

$R^3$  is selected from the group consisting of: lower alkyl, lower cycloalkyl, lower alkylene, aryl or lower aralkyl, all of which optionally mono- or di-substituted with:

- 10 lower alkyl, lower cycloalkyl, haloalkyl, halo, CN, azido, lower alkoxy, (lower alkyl)acyl,  $C_{1-6}$  thioalkyl,  $C_{1-6}$  alkylsulfonyl,  $NHC(O)$ -lower alkyl,  $NHC(O)$ -aryl,  $NHC(O)$ -O-lower alkyl,  $NHC(O)O$ -aryl, aryl, aryloxy, hydroxy, nitro, amino, or Het, said Het optionally mono- or di-substituted with lower alkyl, lower cycloalkyl, lower alkoxy, halo,
- 15 hydroxy, nitrile, trifluoromethyl,  $C(O)R^6$  wherein  $R^6$  is as defined above;

said lower cycloalkyl, aryl, lower aralkyl or Het being optionally fused with a saturated or unsaturated 4 to 6-membered ring optionally containing a heteroatom selected from N, O and S;

and

- 20  $R^4$  is a carboxylic acid, a salt or an ester thereof;

and wherein wavy lines represent bonds of unspecified stereochemistry;

and with the provisos that:

- (1) when A is benzene,  $R^1$  is hydrogen, X and W together form a carbonyl group and Y is 4-methylphenyl, then  $R^3$  cannot be benzyl, 3-fluorophenyl, or 4-nitrophenyl;
- (2) when A is benzene,  $R^1$  is hydrogen, X and W together form a carbonyl group and  $R^3$  is cyclohexyl, then Y cannot be 4-iodophenyl or 4-methylphenyl;
- (3) when A is benzene,  $R^1$  is hydrogen, X and W together form a carbonyl group and Y is 4-fluorophenyl, then  $R^3$  cannot be 4-ethyloxycarbonylphenyl;
- (4) when A is benzene,  $R^1$  is hydrogen, X and W together form a carbonyl group and Y is 2-methylphenyl then  $R^3$  cannot be 4-nitrophenyl;

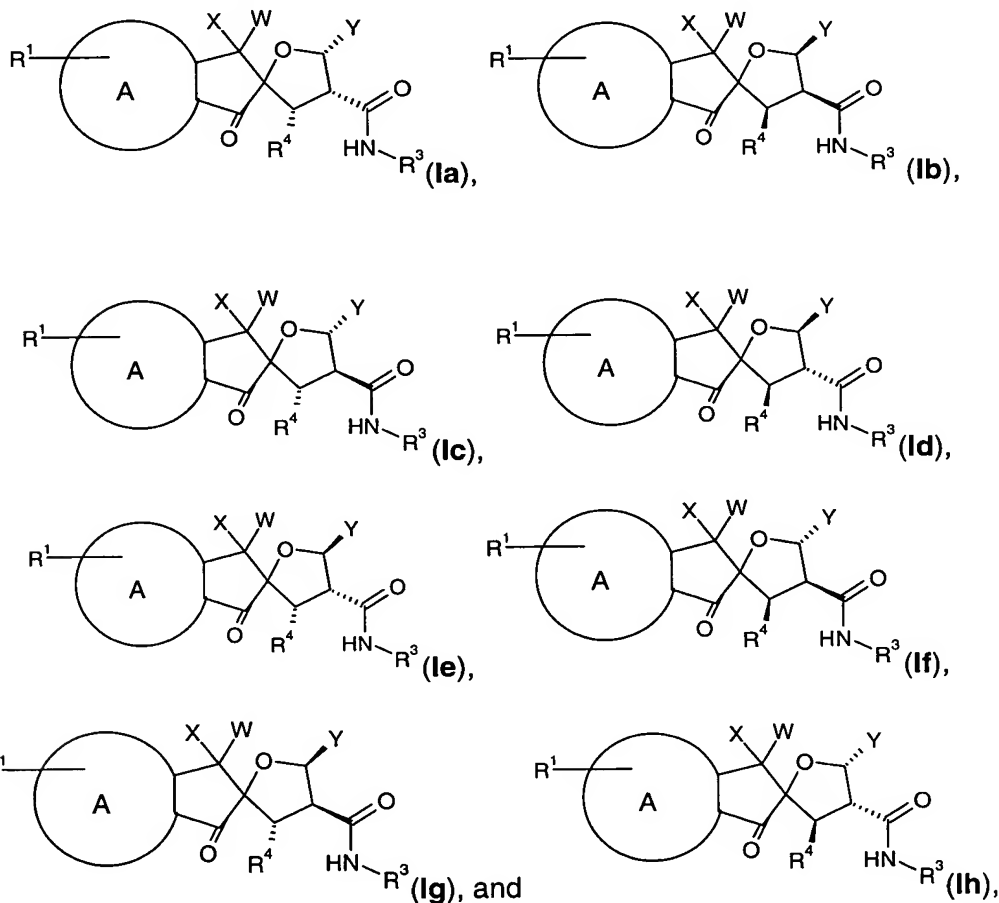
(5) when **A** is benzene, **R**<sup>1</sup> is hydrogen, **X** and **W** together form a carbonyl group and **Y** is 2-methylphenyl, then **R**<sup>3</sup> cannot be phenyl or 2-bromo-4-methylphenyl;

(6) when **A** is benzene, **R**<sup>1</sup> is hydrogen, **X** and **W** together form a carbonyl group and **Y** is 4-chlorophenyl, then **R**<sup>3</sup> cannot be 2-methoxyphenyl or 1,3-benzodioxolyl;

(7) when **A** is benzene, **R**<sup>1</sup> is hydrogen, **X** and **W** together form a carbonyl group and **Y** is 4-ethylphenyl, then **R**<sup>3</sup> cannot be 3-fluorophenyl; and

(8) when **A** is benzene, **R**<sup>1</sup> is hydrogen, **X** and **W** together form a carbonyl group and **Y** is phenyl, then **R**<sup>3</sup> cannot be phenyl.

Alternatively, the first aspect of the invention provides compounds having the following formulae, selected from the group consisting of:



wherein **A**, **X**, **W**, **R**<sup>1</sup>, **Y**, **R**<sup>3</sup> and **R**<sup>4</sup> are as defined above, with the provisos

10



25

Or



(form 3)

20

25

25

25

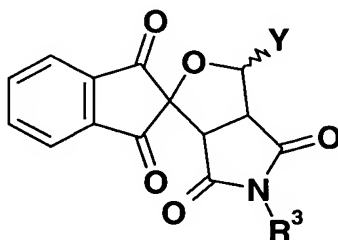
In fourth aspect, the invention provides a method for inhibiting the replication of papillomavirus by exposing virally infected cells to an amount of the compounds of formula (I) inhibiting the papilloma virus E1-E2-DNA complex,  
 5 or a therapeutically acceptable salt or ester thereof, or a composition as described above, (all without the provisos indicated above for formula (I)).

In a fifth aspect, the invention provides a use of compounds of formula (I)  
 10 (without the provisos indicated above for formula (I)) for the manufacture of a medicament for treating a papillomavirus viral infection,.

In an sixth aspect, the invention provides a method of preventing perinatal transmission of HPV from mother to baby, by administering a compound of  
 15 formula (I) (without the provisos indicated above for formula (I)) to the mother prior to giving birth.

In a seventh aspect, the invention provides a use of compounds of formula (I)  
 20 (without the provisos indicated above for formula (I)) for the manufacture of a medicament for preventing perinatal transmission of HPV from mother to baby prior to giving birth.

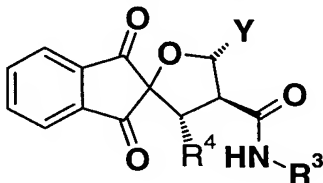
In an eighth aspect, the invention provides an intermediate compound of formula (vi):



vi

wherein Y and R<sup>3</sup> are as defined above, or enantiomers or diastereoisomers thereof, with the provisos indicated above for formula (I).

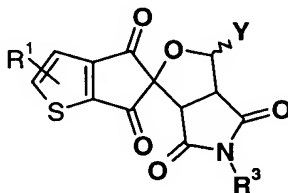
In a ninth aspect, the invention provides an intermediate compound of formula (xx), said compound having trans/trans relative stereochemistry:



xx

wherein Y, R<sup>3</sup>, and R<sup>4</sup> are as defined above, with the provisos indicated above for formula (I).

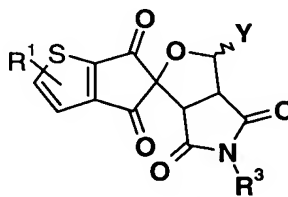
In a tenth aspect, the invention provides an intermediate compound of formula (xxvi):



xxvi

- 5 wherein R<sup>1</sup>, R<sup>3</sup> and Y are as defined above,  
or a salt or an ester thereof, or enantiomers and diastereoisomers thereof,  
without the provisos indicated above for formula (I)..

In a eleventh aspect, the invention provides an intermediate compound of formula (xxxii):

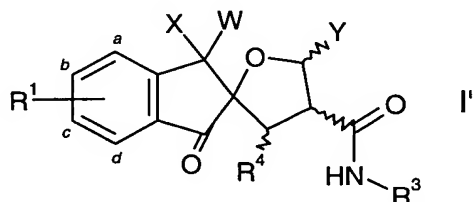


xxxii

wherein R<sup>1</sup>, R<sup>3</sup> and Y are as defined above,

or a salt or an ester thereof, or enantiomers and diastereoisomers thereof, without the provisos indicated above for formula (I).

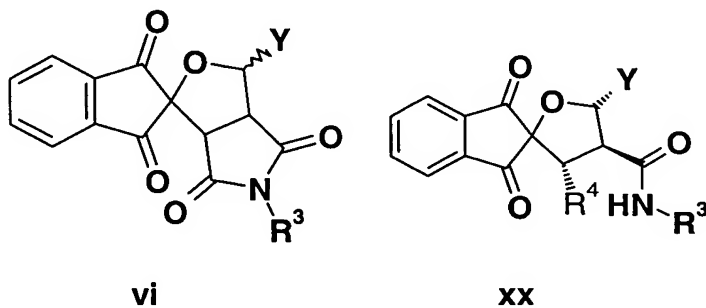
- 5 In a twelfth aspect, the invention provides a process for producing compounds of formula I',



wherein **X**, **R<sup>1</sup>**, **W**, **Y**, **R<sup>3</sup>**, and **R<sup>4</sup>** are as defined above, with the provisos indicated above for formula (I),

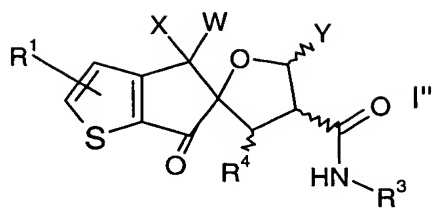
comprising:

- 10 a) hydrolyzing, in a mixture of aqueous base and a co-solvent, either intermediate compound vi or intermediate compound xx



- 15 to produce compounds of formula I', wherein **R<sup>3</sup>**, **R<sup>4</sup>**, and **Y** are as defined above.

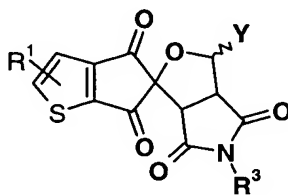
In a thirteenth aspect, the invention provides a process for producing compounds of formula I'',



wherein **X** and **W** together form a carbonyl group, **R<sup>4</sup>** is a carboxylic acid or an ester, and **R<sup>1</sup>**, **Y**, and **R<sup>3</sup>** are as defined above, without the provisos indicated above for formula (I),

5 comprising:

a) hydrolyzing, in a mixture of aqueous base and a co-solvent, intermediate compound xxvi,

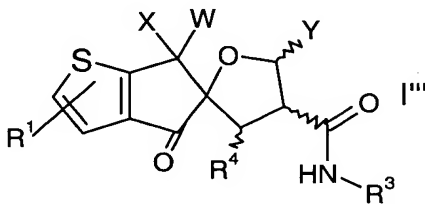


xxvi

so as to produce compounds of formula I'', wherein **R<sup>1</sup>**, **Y**, and **R<sup>3</sup>** are as defined above.

10

In a fourteenth aspect, the invention provides, a process for producing compounds of formula I''',

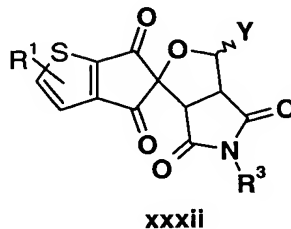


15

wherein **X** and **W** together form a carbonyl group, **R<sup>4</sup>** is a carboxylic acid or an ester, and **R<sup>1</sup>**, **Y**, and **R<sup>3</sup>** are as defined above, without the provisos indicated above for formula (I),

comprising:

- a) hydrolyzing, in a mixture of aqueous base and a co-solvent, intermediate compound xxxii



- 5 so as to produce compounds of formula I''', wherein R<sup>1</sup>, Y, and R<sup>3</sup> are as defined above.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

### Definitions

- 10 As used herein, the following definitions apply unless otherwise noted:

The term "halo" as used herein means a halo radical selected from bromo, chloro, fluoro or iodo.

- 15 The term "lower alkyl" (or C<sub>1-6</sub> alkyl) as used herein, either alone or in combination with another radical, means straight or branched-chain alkyl radicals containing up to six carbon atoms and includes methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl and 1,1-dimethylethyl. The term "C<sub>0-6</sub> alkyl" preceding a radical means that this radical  
 20 can optionally be linked through a C<sub>1-6</sub> alkyl radical or the alkyl may be absent (C<sub>0</sub>).

- The term "lower cycloalkyl" as used herein, either alone or in combination with another radical, means saturated cyclic hydrocarbon radicals containing from  
 25 three to seven carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

The term "lower alkoxy" as used herein means straight chain alkoxy radicals containing one to four carbon atoms and branched chain alkoxy radicals containing three to four carbon atoms and includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter radical is known commonly as tert-butoxy.

The term "haloalkyl" as used herein means alkyl radical containing one to six carbon atoms wherein one or more hydrogen atom is replaced by a halogen atom (e.g. trifluoromethyl).

10

The term "amino" as used herein means an amino radical of formula  $\text{-NH}_2$ .

The term "lower alkylamino" as used herein means alkylamino radicals containing one to six carbon atoms and includes methylamino, propylamino, (1-methylethyl)amino and (2-methylbutyl)amino. The term "di(lower alkyl)amino" means an amino radical having two lower alkyl substituents each of which contains one to six carbon atoms and includes dimethylamino, diethylamino, ethylmethylamino and the like.

The term "acyl" as used herein, either alone or in combination with another radical, refers to groups  $\text{-C(O)R}$ , wherein R is lower alkyl or lower alkoxy.

The term " $\text{C}_6$  or  $\text{C}_{10}$  aryl" as used herein, either alone or in combination with another radical, means either an aromatic monocyclic system containing 6 carbon atoms or an aromatic cyclic system containing 10 carbon atoms. For example, aryl includes phenyl or naphthalene.

The term " $\text{C}_{7-16}$  aralkyl" as used herein, either alone or in combination with another radical, means an aryl as defined above linked through an alkyl group, wherein alkyl is as defined above containing from 1 to 6 carbon atoms. Aralkyl includes for example benzyl, and butylphenyl.

The term "Het" as used herein means a monovalent radical derived by removal of a hydrogen from a five- or six-membered, saturated or unsaturated heterocycle containing from one to three heteroatoms selected from nitrogen, oxygen and sulfur. Optionally, the heterocycle may bear one or two substituents; for example, N-oxido, lower alkyl, (C<sub>1-3</sub>)alkyl-phenyl, lower alkoxy, halo, amino or lower alkylamino. Again optionally, the five- or six-membered heterocycle can be fused to a second cycloalkyl, an aryl (e.g. phenyl) or another heterocycle.

Examples of suitable heterocycles and optionally substituted heterocycles include morpholine, thiadiazole, quinoline, 3,4-methylene-dioxyphenyl, benzothiazole, pyrrolidine, tetrahydrofuran, thiazolidine, pyrrole, 1H-imidazole, 1-methyl-1H-imidazole, pyrazole, furan, thiophene, oxazole, isoxazole, thiazole, 2-methylthiazole, 2-aminothiazole, 2-(methylamino)-thiazole, piperidine, 1-methylpiperidine, 1-methylpiperazine, 1,4-dioxane, pyridine, pyridine N-oxide, pyrimidine, 2,4-dihydroxypyrimidine, 2,4-dimethylpyrimidine, 2,6-dimethylpyridine, 1-methyl-1H-tetrazole, 2-methyl-2H-tetrazole, benzoxazole and thiazolo[4,5-b]-pyridine.

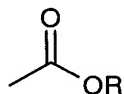
The term "pharmaceutically acceptable carrier" as used herein means a non-toxic, generally inert vehicle for the active ingredient which does not adversely affect the ingredient.

The term "effective amount" means a predetermined antiviral amount of the antiviral agent, i.e. an amount of the agent sufficient to be effective against the virus *in vivo*.

The compounds of formula (I) can be obtained in the form of therapeutically acceptable salts. The term "pharmaceutically acceptable salt" as used herein includes those derived from pharmaceutically acceptable bases. Examples of suitable bases include choline, ethanolamine and ethylenediamine. Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>++</sup> salts are also contemplated to be within the scope of the invention (also see Pharmaceutical salts, Birge, S.M. et al., J. Pharm. Sci. (1977), 66, 1-

19, incorporated herein by reference).

The term "pharmaceutically acceptable ester" as used herein, either alone or in combination with another radical, means esters of the compound of formula (I) in which the carboxyl function is replaced by an alkoxycarbonyl function:



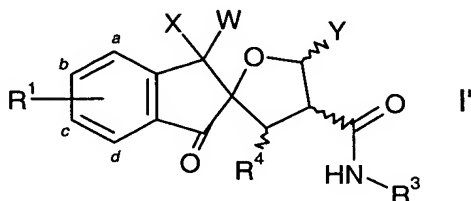
in which the **R** moiety of the ester is selected from alkyl (e.g. methyl, ethyl, n-propyl, t-butyl, n-butyl); alkoxyalkyl (e.g. methoxymethyl); alkoxyacyl (e.g. acetoxymethyl); aralkyl (e.g. benzyl); aryloxyalkyl (e.g. phenoxymethyl); aryl (e.g. phenyl), optionally substituted with halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy. Other suitable prodrug esters can be found in Design of prodrugs, Bundgaard, H. Ed. Elsevier (1985) incorporated herewith by reference. Such pharmaceutically acceptable esters are usually hydrolyzed *in vivo* when injected in a mammal and transformed into the acid form of the compound of formula (I).

With regard to the esters described above, unless otherwise specified, any alkyl moiety present advantageously contains 1 to 16 carbon atoms, particularly 1 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group.

In particular the esters may be a C<sub>1-16</sub> alkyl ester, an unsubstituted benzyl ester or a benzyl ester substituted with at least one halogen, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkoxy, nitro or trifluoromethyl.

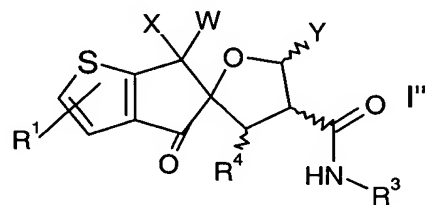
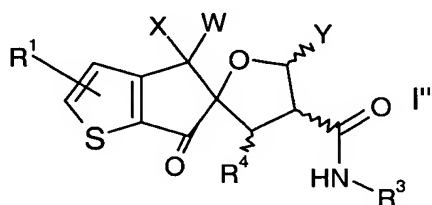
## Preferred embodiments

According to a first embodiment of this invention, preferably compounds of the invention are those in which ring **A** is a benzene ring, as represented by the formula I':



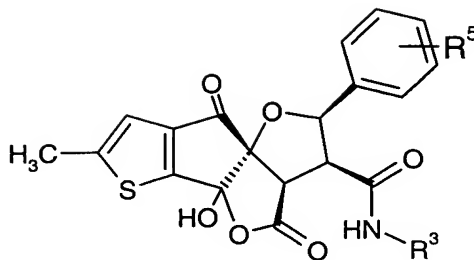
Wherein **X**, **W**, **R<sup>1</sup>**, **Y** and **R<sup>3</sup>** are as defined above, with the provisos indicated above for formula (I). The compounds of formula I' exist in forms (1), (2) and (3), as described for the compounds of formula I.

- 10 Alternatively preferably, compounds of this invention are those in which ring **A** is a five-membered ring containing a sulfur atom, as represented by the formulae I'' and I''':



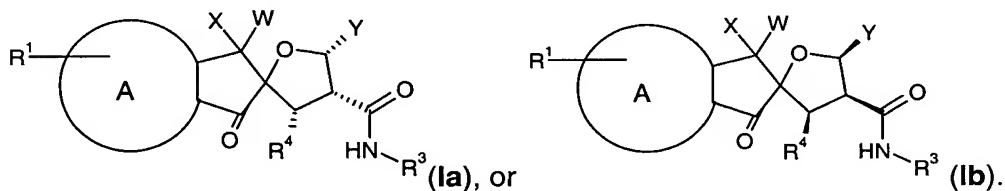
- 15 Wherein **X**, **W**, **R<sup>1</sup>**, **Y** and **R<sup>3</sup>** are as defined above, without the provisos indicated above for formula (I). The compounds of formulae I'' and I''' exist in forms (1), (2) and (3), as described for the compounds of formula I.

Alternatively even more preferably, compounds of the invention have the following formula:

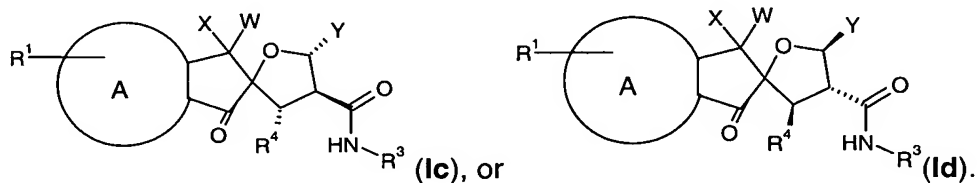


wherein  $R^3$  and  $R^5$  are as defined above, without the provisos indicated above for formula (I).

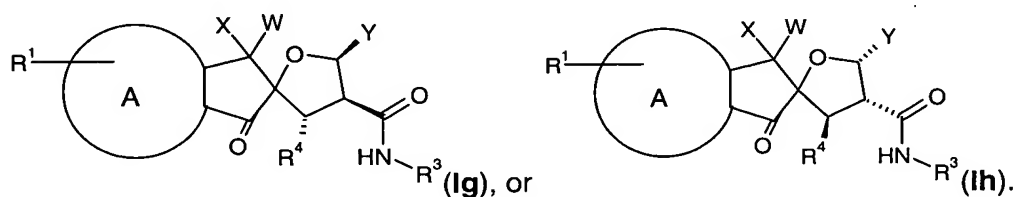
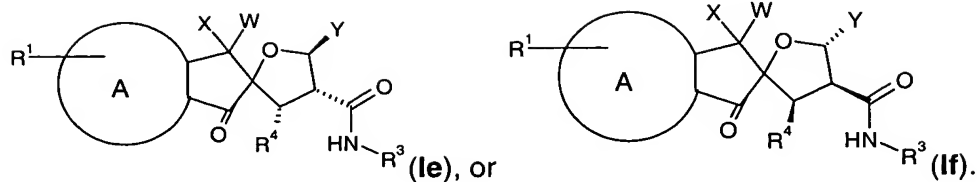
- 5 The compounds of the present invention can be synthesized as racemic mixtures and then separated in their respective single diastereoisomers. All such diastereoisomers are contemplated within the scope of the present invention.
- 10 Preferably, such diastereoisomers include mixture of compounds with the following relative stereochemistry between  $[Y \text{ \& } C(O)NH-R^3]$  and  $[C(O)NH-R^3 \text{ \& } R^4]$ :



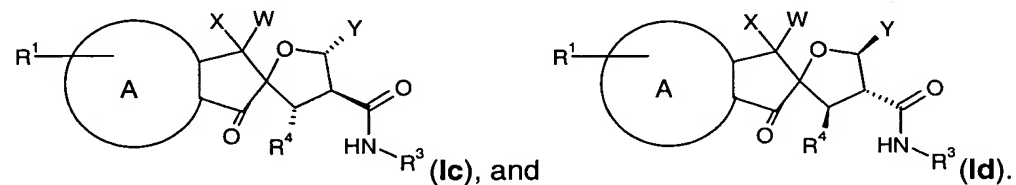
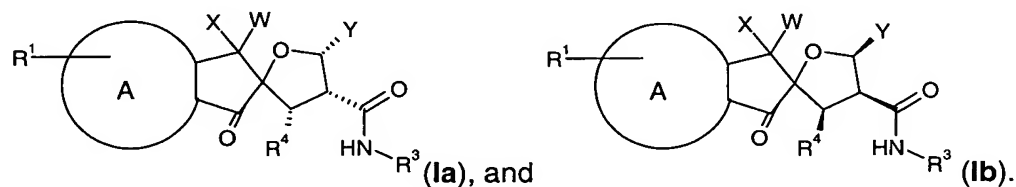
Formulas (Ia) and (Ib) both represent racemic mixtures of compounds with the relative stereochemistry referred to as "cis/cis".

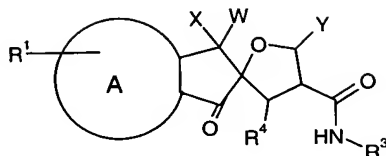


Formulas (Ic) and (Id) both represent racemic mixtures of compounds with the relative stereochemistry referred to as "trans/trans".

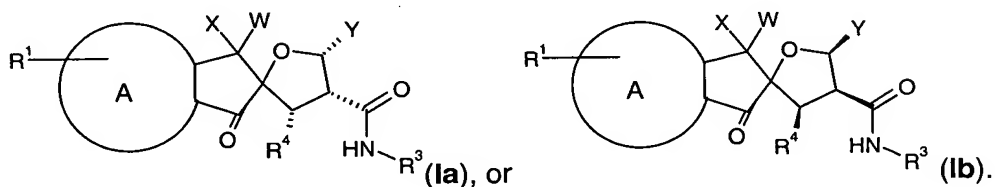


More preferably, such diastereoisomers include mixture of compounds with the relative stereochemistry "*cis/cis*":



racemic mixture of **Ia** and **Ib**

- 5 Still most preferably, the invention comprises pure enantiomers of compounds of formula (**Ia**) or (**Ib**) with the relative stereochemistry "*cis/cis*":



- 10 With respect to compounds of formulae **I'**, **I''**, **I'''**, **Ia**, **Ib**, **Ic**, **Id**, **Ie**, **If**, **Ig**, and **Ih**, preferably **X** is H and **W** is OH; or **X** and **W** form a carbonyl group. Most preferably, **X** and **W** form a carbonyl group.

With respect to compounds of the formulae **I'**, **I''** and **I'''** preferably **A** is phenyl or thiophene. Most preferably, **A** is thiophene.

- 15 With respect to compounds of the formulae **I'**, **I''** and **I'''** preferably **R**<sup>1</sup> is H; or one or two substituents independently selected from the group consisting of: hydroxy; halo; lower alkyl; lower alkoxy; lower thioalkyl; haloalkyl (e.g. trifluoromethyl); or -C(O)**R**<sup>2</sup> wherein **R**<sup>2</sup> is lower alkyl, aryloxy or benzyloxy.

- 20 More preferably, **R**<sup>1</sup> is H, halo or C<sub>1-4</sub> alkyl.

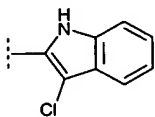
Even more preferably, **R**<sup>1</sup> is H, fluoro or methyl.

Most preferably, **R**<sup>1</sup> is H or methyl.

Preferably, **Y** is phenyl optionally mono- or di-substituted with  $R^5$  or  $C(O)R^6$ , wherein  $R^5$  is lower alkyl, lower cycloalkyl, lower alkoxy, halo, hydroxy, nitrile or trifluoromethyl, and  $R^6$  is lower alkyl, lower cycloalkyl, lower alkoxy, hydroxy or trifluoromethyl; said phenyl ring being optionally fused with a saturated or unsaturated 4 to 6-membered ring optionally containing a heteroatom selected from N, O and S; or **Y** is ethylene-phenyl, said ethylene moiety being optionally mono-substituted with lower alkyl, wherein said phenyl ring is optionally mono- or di-substituted with  $R^5$  or  $C(O)R^6$ , wherein  $R^5$  and  $R^6$  are as defined above; said phenyl ring being optionally fused with a saturated or unsaturated 4- to 6-membered ring optionally containing a heteroatom selected from N, O and S.

More preferably, **Y** is naphthyl,  $CH=CH$ -phenyl,  $C(CH_3)=CH$ -phenyl or phenyl, wherein the phenyl ring is optionally mono- or di-substituted at the 3, 4, or 5 position with  $R^5$ , wherein  $R^5$  is halo,  $C_{1-4}$  alkyl, hydroxy,  $CF_3$  or  $NHC(O)$ -(lower alkyl).

Still more preferably, **Y** is phenyl optionally substituted with: 3,4-Cl; 3-F,4-Cl; 3-Cl,4-F; 3,4-Br; 3-F,4- $CH_3$ ; 3,4- $CH_3$ ; 3- $CF_3$ ,  $NHC(O)$ -( $CH_2$ ) $_3$  $CH_3$  and



Most preferably, **Y** is phenyl optionally substituted with:

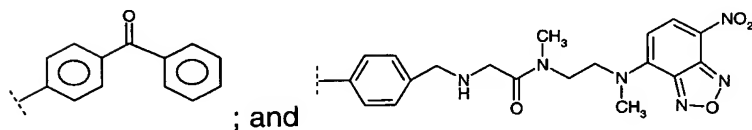
3,4-Cl and 3,4-Br.

Preferably,  $R^3$  is selected from the group consisting of:

cyclohexyl;  $C_{1-6}$  alkyl;  $C_{1-6}$  thioalkyl; ( $C_{1-6}$  alkyl)phenyl wherein the phenyl ring is optionally substituted with:

lower alkyl,  $CF_3$ , halo, CN, azido, lower alkoxy, (lower alkyl)acyl,  $C_{1-6}$

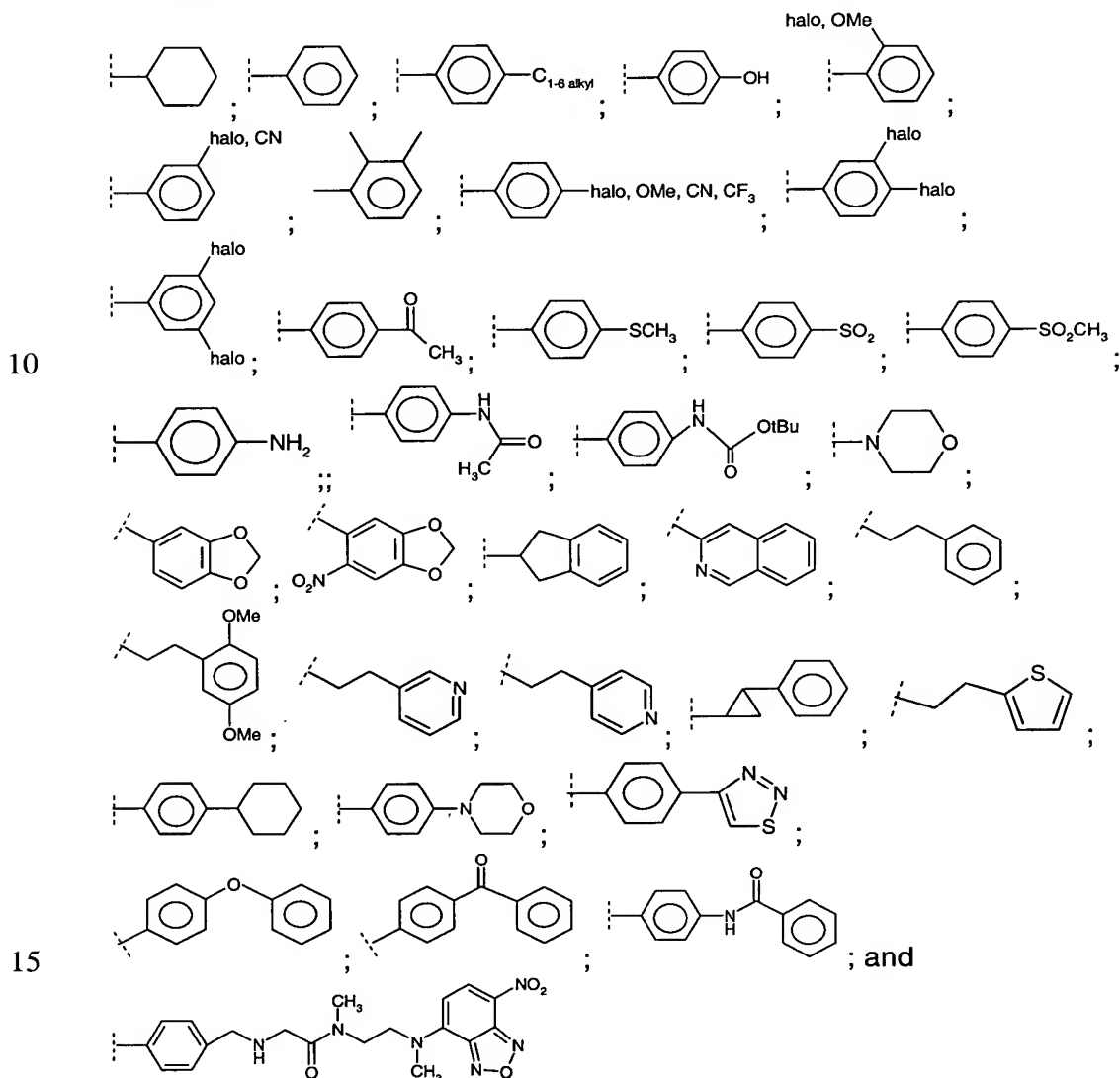
thioalkyl, C<sub>1-6</sub> alkylsulfonyl, NHC(O)-lower alkyl, aryl, aryloxy, hydroxy, nitro, amino, or Het, said Het optionally mono- or di-substituted with lower alkyl, lower alkoxy, halo, hydroxy, nitrile, trifluoromethyl;



5

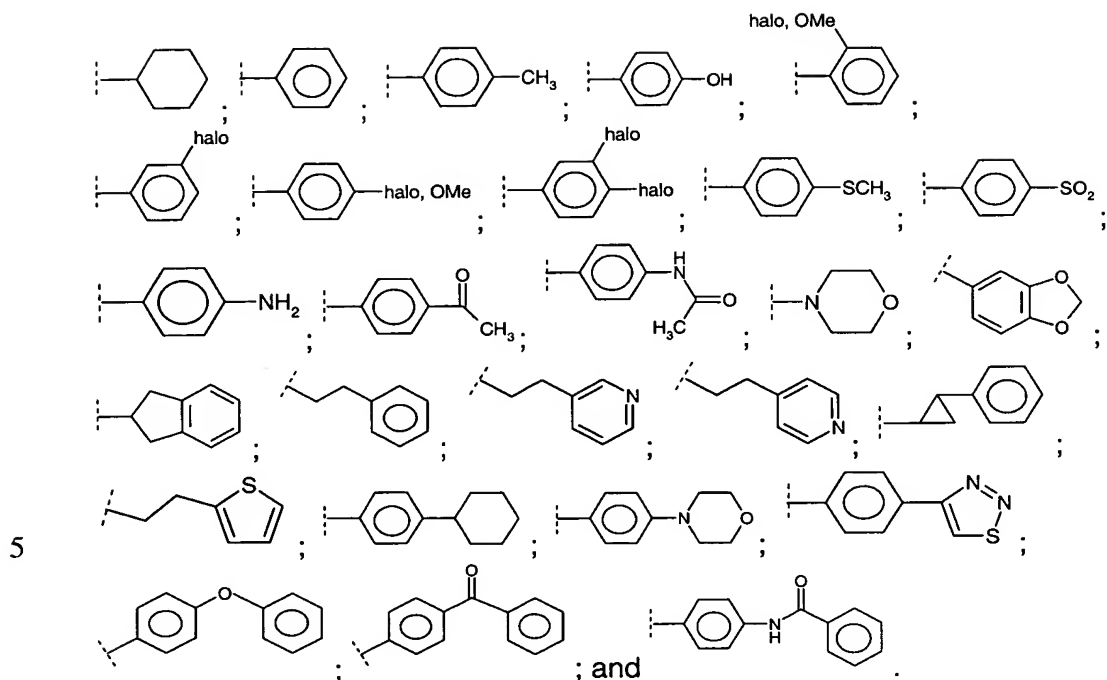
Even more preferably, **R<sup>3</sup>** is selected from the group consisting of:

C<sub>1-6</sub> alkyl; C<sub>1-6</sub> thioalkyl;



15

Most preferably, **R<sup>3</sup>** is selected from the group consisting of:

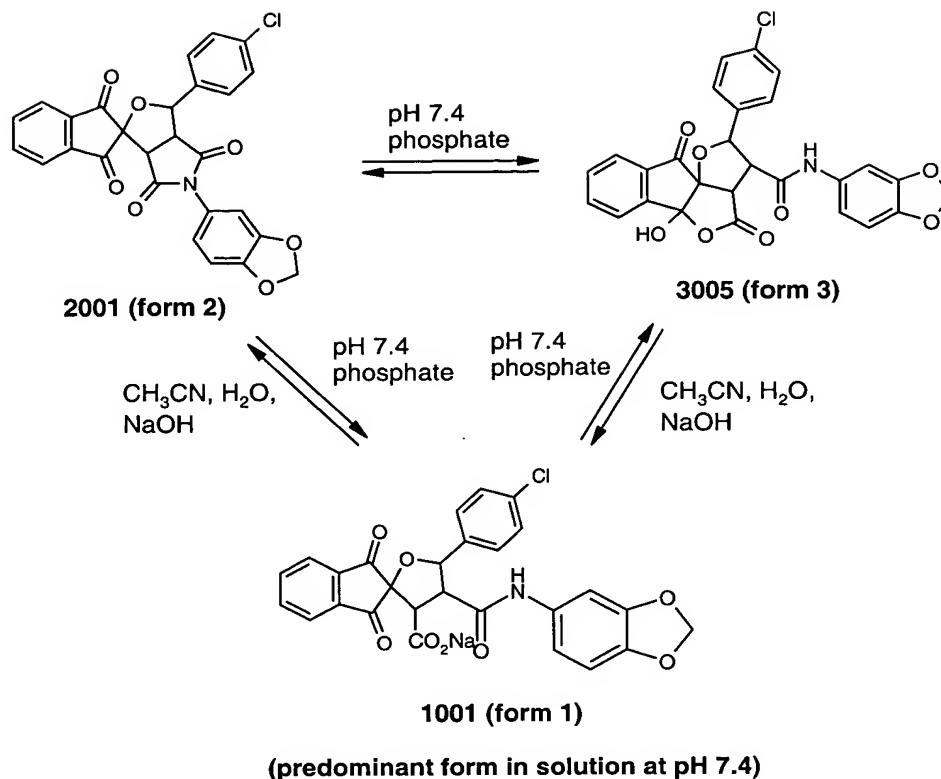


Particularly preferred compounds of the invention are compounds having the formula I". Of compounds having the formula I", those having the "cis/cis" configuration are particularly preferred.

Preferably, R<sup>4</sup> is a carboxylic acid, a salt or an ester thereof.

#### Different forms of compounds of formula (I).

Compounds of formula (I) according to the invention can present themselves in different forms according to the solvent and the pH in which they are dissolved. For example, compound **1001** (Table 1, form 1) can exist in equilibrium with compound **2001** (see Table 2, hereinafter) and compound **3005** (Table 3) in form (3) when dissolved in phosphate buffer at pH 7.4. Without wishing to be bound by theory, it is believed that the predominant form in solution at pH 7.4 is represented by form (1).



### Specific embodiments

- 5 Included within the scope of this invention are all compounds of formula  
 formulae I, I', I'', I''', Ia, Ib, I,c, Id, Ie, If, Ig, or Ih, as presented in Tables 1 to  
 10 (with the exception of those compounds excluded by provisos).

### Anti-papilloma Virus Activity

- 10 The antiviral activity of the compounds of formula (I) can be demonstrated by  
 biochemical and biological procedures showing the inhibitory effect of the  
 compounds on DNA replication.

- 15 Preferably, the compounds of formula (I) as described above are inhibitory  
 against human papillomavirus (HPV). More preferably the compounds are  
 active against HPV low risk or high risk type. Even more preferably, against  
 low risk type HPV (i.e. type 6, type 11 and type 13, and especially HPV type  
 11). Alternatively, the high-risk type is selected from the group consisting of  
 types 16, 18, 31, 33, 35, 45, 52, or 58, preferably, type 16). Most preferably,

the compounds of the invention are directed against HPV types 6 and 11, even most preferably, against HPV-11.

5 A biochemical procedure for demonstrating anti-papilloma virus activity for the compounds of formula (I) is described in the examples hereinafter. This particular assay determines the ability of a test compound to inhibit the activity (IC<sub>50</sub>) of HPV-11 DNA replication. More specifically, in the assay described herein, the inhibitory activity of the test compound is evaluated based on its ability to interfere with the E1-E2-DNA origin of replication interaction, thereby  
10 inhibiting initiation of viral DNA replication.

Methods for demonstrating the inhibitory effect of the compounds of formula (I) on papilloma viral replication involving *in vitro* assays are described in Examples 11 to 15 herein.

15 When a compound of formula (I), or one of its therapeutically acceptable salts, is employed as an antiviral agent, it may be administered orally, topically or systemically to mammals, e.g. humans, rabbits or mice, alone or in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound,  
20 chosen route of administration and standard biological practice.

Whether it be termed treatment or prophylaxis, a compound of formula (I) may also be used to prevent perinatal transmission of HPV from mother to baby, by  
25 administration to the mother prior to giving birth. More specifically, a compound of formula (I) may be used to prevent laryngeal papillomatosis in the baby.

For oral administration, the compound or a therapeutically acceptable salt  
30 thereof can be formulated in unit dosage forms such as capsules or tablets each containing a predetermined amount of the active ingredient, ranging from about 25 to 500 mg, in a pharmaceutically acceptable carrier.

For topical administration, the compound may be formulated in pharmaceutically accepted vehicles containing 0.1 to 5 percent, preferably 0.5 to 5 percent, of the active agent. Such formulations can be in the form of a solution, cream or lotion.

For parenteral administration, the compound of formula (I) may be administered by either intravenous, subcutaneous or intramuscular injection, in compositions with pharmaceutically acceptable vehicles or carriers. For administration by injection, it is preferred to use the compounds in solution in a sterile aqueous vehicle which may also contain other solutes such as buffers or preservatives as well as sufficient quantities of pharmaceutically acceptable salts or of glucose to make the solution isotonic.

Suitable vehicles or carriers for the above noted formulations are described in standard pharmaceutical texts, e.g. in "Remington's The Science and Practice of Pharmacy", 19th ed., Mack Publishing Company, Easton, Penn., 1995, or in "Pharmaceutical Dosage Forms And Drugs Delivery Systems", 6th ed., H.C. Ansel et al., Eds., Williams & Wilkins, Baltimore, Maryland, 1995.

The dosage of the compound will vary with the form of administration and the particular active agent chosen. Furthermore, it will vary with the particular host under treatment. Generally, treatment is initiated with small increments until the optimum effect under the circumstance is reached. In general, the compound of formula I is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

For oral administration, the compound or a therapeutically acceptable salt may be administered in the range of 10 to 200 mg per kilogram of body weight per day, with a preferred range of 25 to 150 mg per kilogram.

For topical application, the compound of formula (I) may be administered in a suitable formulation to the infected area of the body e.g. the skin, the genitalia, in an amount sufficient to cover the infected area. The treatment may be repeated, for example, every four to six hours until lesions heal.

5

For systemic administration, the compound of formula (I) may be administered at a dosage of 10 mg to 150 mg per kilogram of body weight per day, although the aforementioned variations will occur. However, a dosage level that is in the range of from about 10 mg to 100 mg per kilogram of body weight per day is most desirably employed in order to achieve effective results.

10

Although the formulations disclosed herein are indicated to be effective and relatively safe medications for treating papilloma viral infections, the possible concurrent administration of these formulations with other medications or agents to obtain beneficial results is also contemplated. Such other medications or agents include TCA, podophyllin, podofilox, Interferon or Imiquimod.

15

In addition to the above-mentioned antiviral agents, the compounds according to the invention may also be used post-cryotherapy or post-surgery or in combination with any other treatment for physically removing warts.

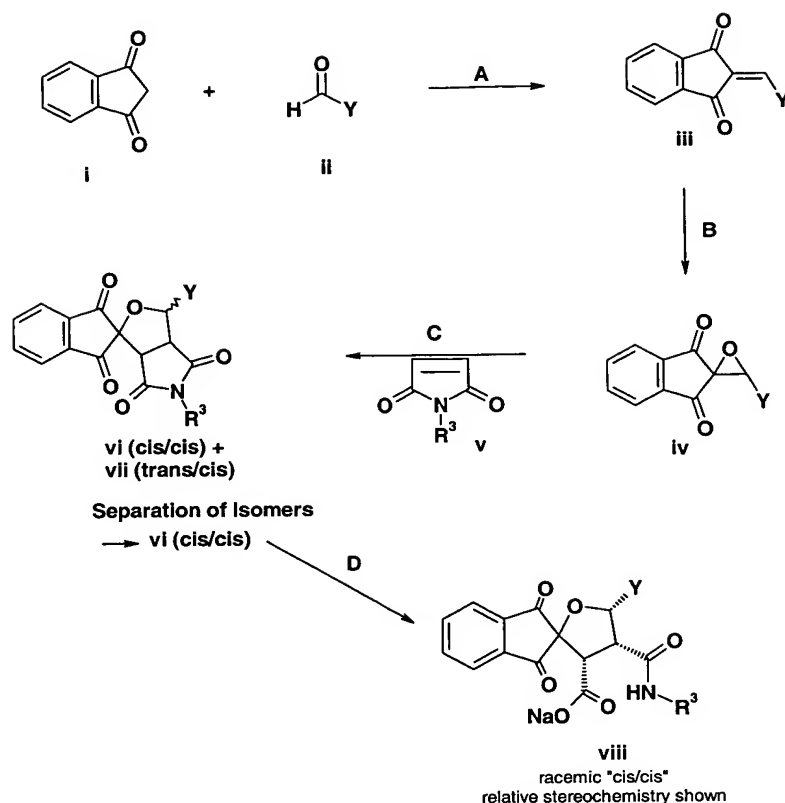
20

### Methodology and Synthesis

25

The synthesis of compounds of formula I' is illustrated in Scheme I. The radicals  $Y$ ,  $R^3$  and  $R^4$  are as defined previously:

Scheme I



A): Commercially available indan-1, 3-dione (i) [or prepared according to known literature procedure: D.R. Bukle, N.J. Morgan, J.W. Ross, H. Smith, B.A. Spicer; J. Med. Chem. 1973, 16, 1334-1339] is condensed with aldehyde (ii) in a protic solvent (e.g. ethanol or propanol) in the presence of a catalytic amount of an organic amine (e.g. piperidine) to form the benzylidene (iii).

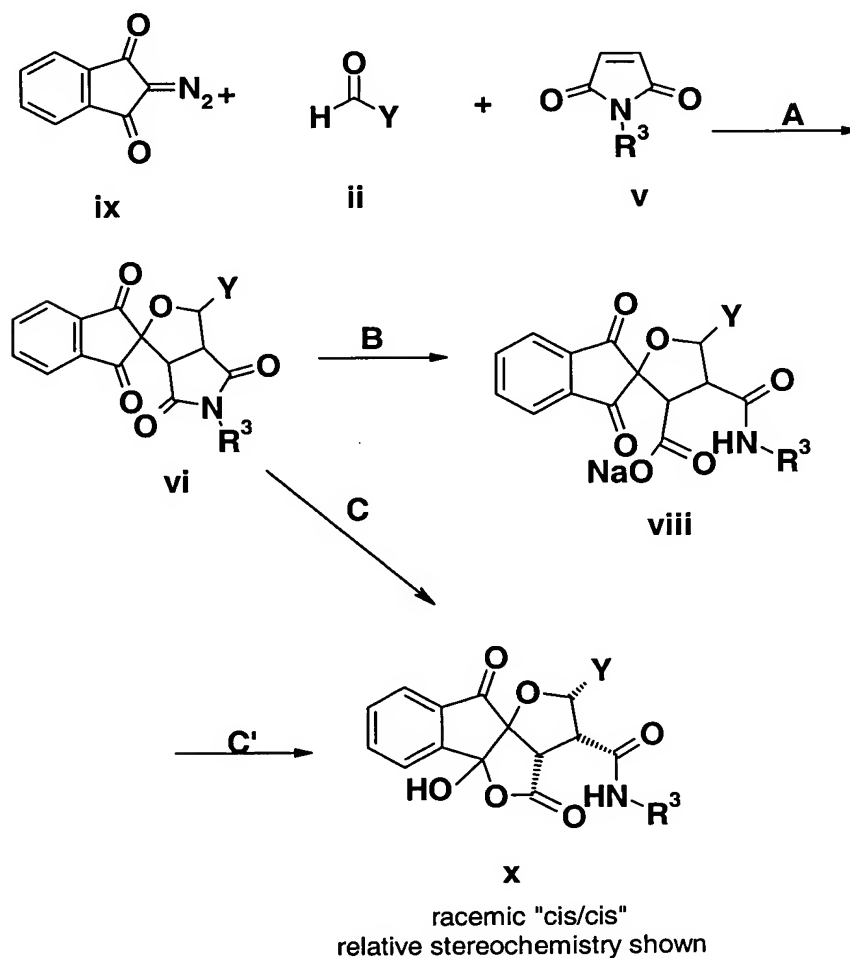
B): Benzylidene (iii) is converted to the epoxide (iv) by base-catalyzed oxidation with hydrogen peroxide in a protic solvent (such as methanol).

C): Epoxide (iv) undergoes thermal 1,3-dipolar cycloaddition in the presence of maleimide (v) at temperatures ranging from 80 to 100 °C in a solvent such as toluene or xylene (ref.: M.Y. Krysin, I.K. Anohina, L.P. Zalukaev; Khimiya Geterotsiklicheskikh Soedinenii, 1987, 11, 1463-1466). Thus racemic "cis/cis" (vi) and racemic "cis/trans" (vii) are obtained after purification (crystallization, flash column chromatography, or preparative HPLC). In general maleimides

such as (v) are commercially available or alternatively can be easily prepared using literature procedures (e.g. P.Y. Reddy, S. Kondo, T. Toru, Y. Ueno; J. Org. Chem., 1997, 62, 2652-2654).

- 5 D): Racemic "*cis/cis*" compound (vi) is hydrolyzed to yield its opened carboxylate form (viii) also as "*cis/trans*" racemic mixture. Hydrolysis is achieved under aqueous basic conditions, such as aqueous sodium hydroxide and acetonitrile as a co-solvent.
- 10 Alternatively steps A) and B) can be carried out as a "one pot" reaction using an appropriate solvent (e.g. propanol) as described in Scheme II:

Scheme II



A): Diazoindan-1, 3-dione (ix) [prepared according to literature procedure: J. Chem. Soc. Chem. Commun., 1990, 652-653] was reacted with aldehyde (ii) and maleimide (v) in the presence of a catalytic amount of rhodium(II) to give racemic "*cis/cis*" compound (vi).

5

B): The corresponding carboxylate (viii) is made following the hydrolysis procedure described in Scheme I, step D).

10

C) This step presents a further method for synthesizing compounds of formula I' in an alternative closed form. Racemic "*cis/cis*" compound (vi) is first hydrolyzed using procedure described in Scheme I, step D, followed with treatment with acid using dilute aqueous HCl to produce hydroxylactone (x) as racemic "*cis/cis*".

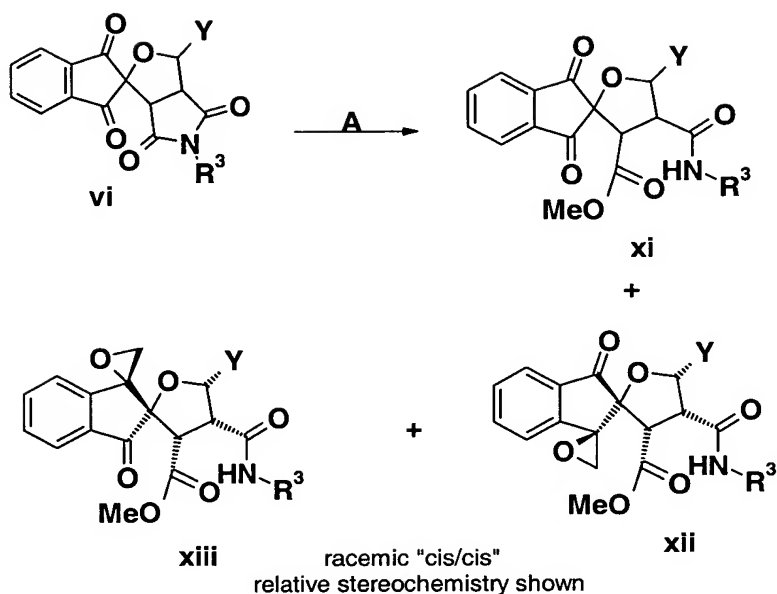
15

C') Alternatively the sodium salt intermediate (viii) is passed through a reverse phase column (HPLC) using a trifluoroacetic acid containing eluent to yield the hydroxylactone (x).

20

Compounds of formula I' wherein **X** and **W** form an epoxide are synthesized as illustrated in Scheme III:

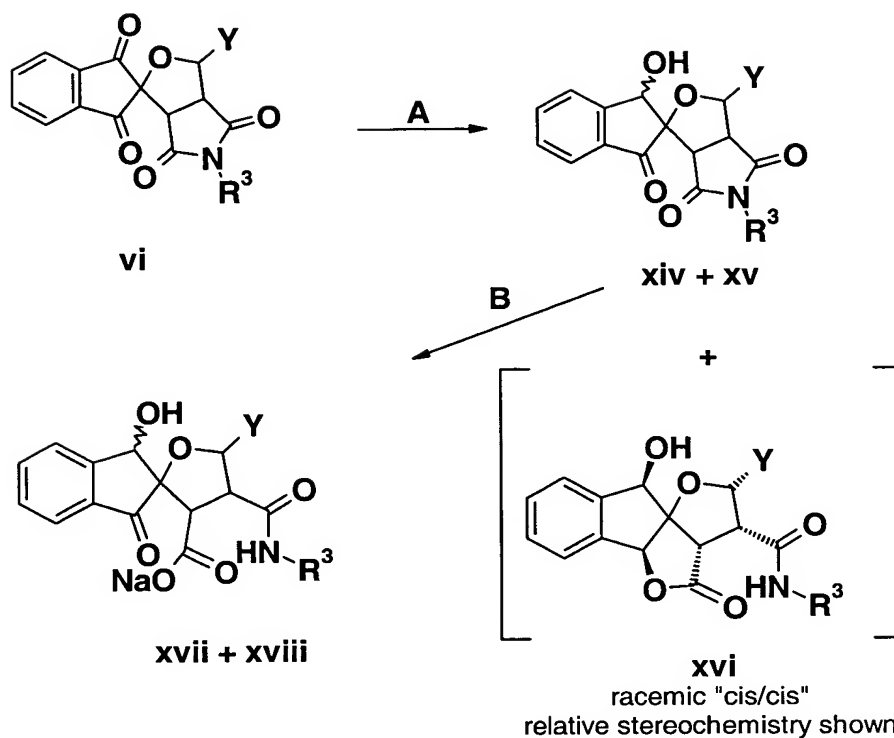
Scheme III



- 5 A): Racemic "*cis/cis*" compound (vi) is converted to the desired inhibitors via first hydrolysis under basic conditions, following by acidification and treatment with diazomethane. Compounds (xi), (xii) and (xiii) are separated from the mixture by flash chromatography or by preparative HPLC.

- 10 Scheme IV illustrates a general method for the synthesis of compounds of formula I' wherein **X** is H and **W** is hydroxy:

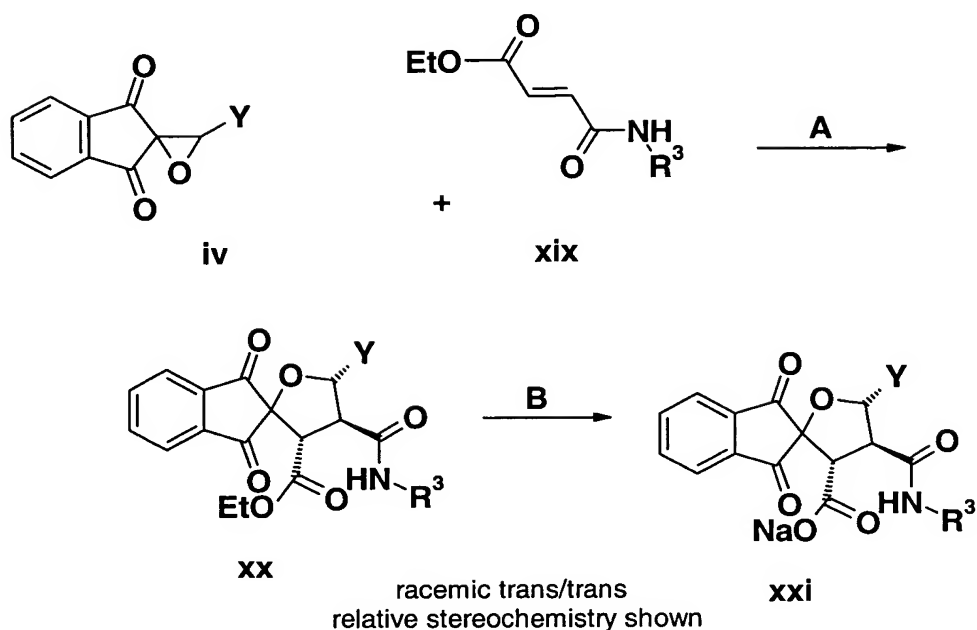
Scheme IV



- 5 A): Reduction of racemic "cis/cis" compound (vi) is achieved using a hydride source (e.g. sodium borohydride) to give mixtures of the monohydroxy derivatives (xiv and xv) in addition to the hydroxy lactone (xvi) having the relative stereochemistry as shown.
- 10 B): After separation, racemic (xiv and xv) are hydrolyzed using the same procedure as in Scheme I, step D) to give racemic (xvii and xviii) after preparative separation.

Scheme V illustrates the method for synthesizing compounds of formula I' with the relative stereochemistry in *trans/trans*.

Scheme V

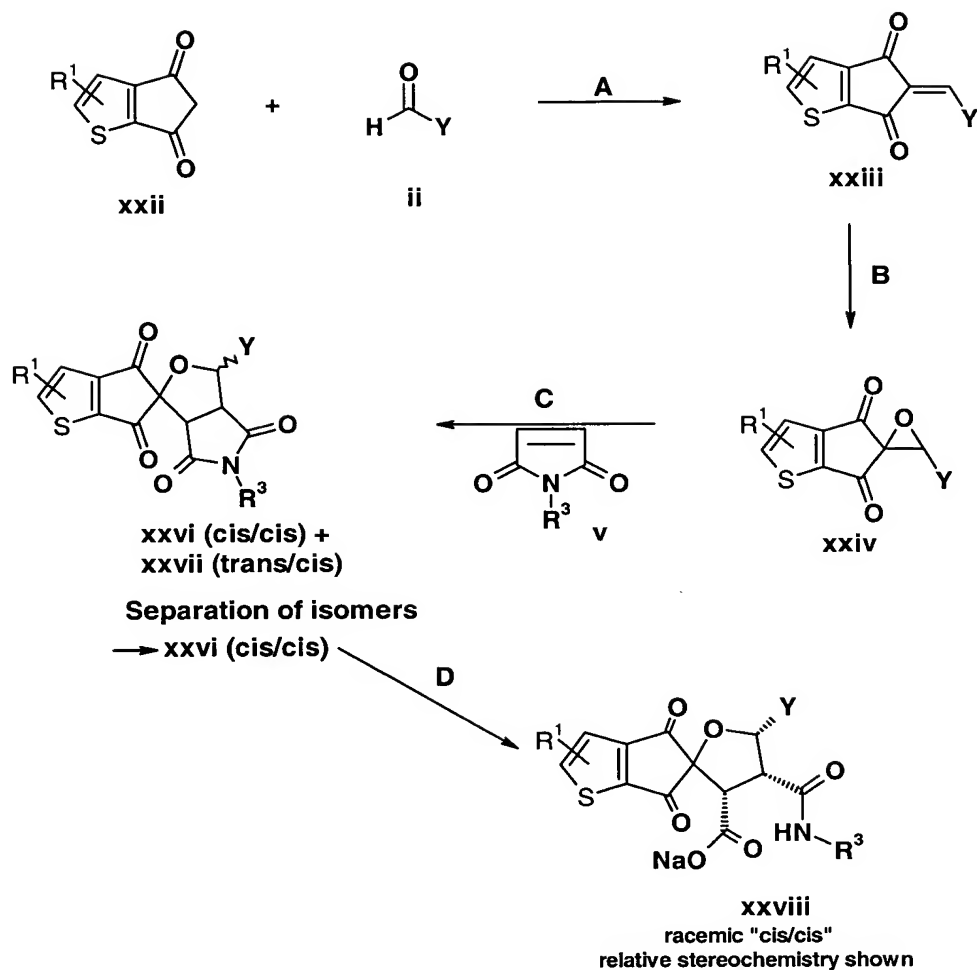


5 A): The amide (xix) obtained using literature procedure (ex: G. B. Villeneuve and T. H. Chan *Tetrahedron Letters*, **1997**, 38,6484) is reacted with epoxide (iv) in toluene under refluxing conditions to yield the cycloadduct ester (xx) as racemic *trans/trans* isomers.

10 B): Hydrolysis of the ester (xx) is done as described in Scheme I, step D) to give the desired carboxylate (xxi) also as racemic *trans/trans* isomers.

Compounds of formula I'' and of formula I''', may be made in an analogous manner to those of formula I', except that instead of indan-1, 3-dione as starting material, compound xxii is used.

## Scheme VI



5

A): Compound (xxii) [prepared by homologation of commercially available 5-methyl-2-thiophenecarboxaldehyde with malonic acid, followed by reduction of the exocyclic double bond with sodium amalgam, or hydrogen over palladium, followed by cyclization with oxalyl chloride/ $\text{AlCl}_3$ , or polyphosphoric acid, followed by oxidation with  $\text{CrO}_3/t$ -butylhydroperoxide] is condensed with aldehyde (ii) in a protic solvent (e.g. ethanol or propanol) in the presence of a catalytic amount of an organic amine (e.g. piperidine) to form the benzylidene (xxiii).

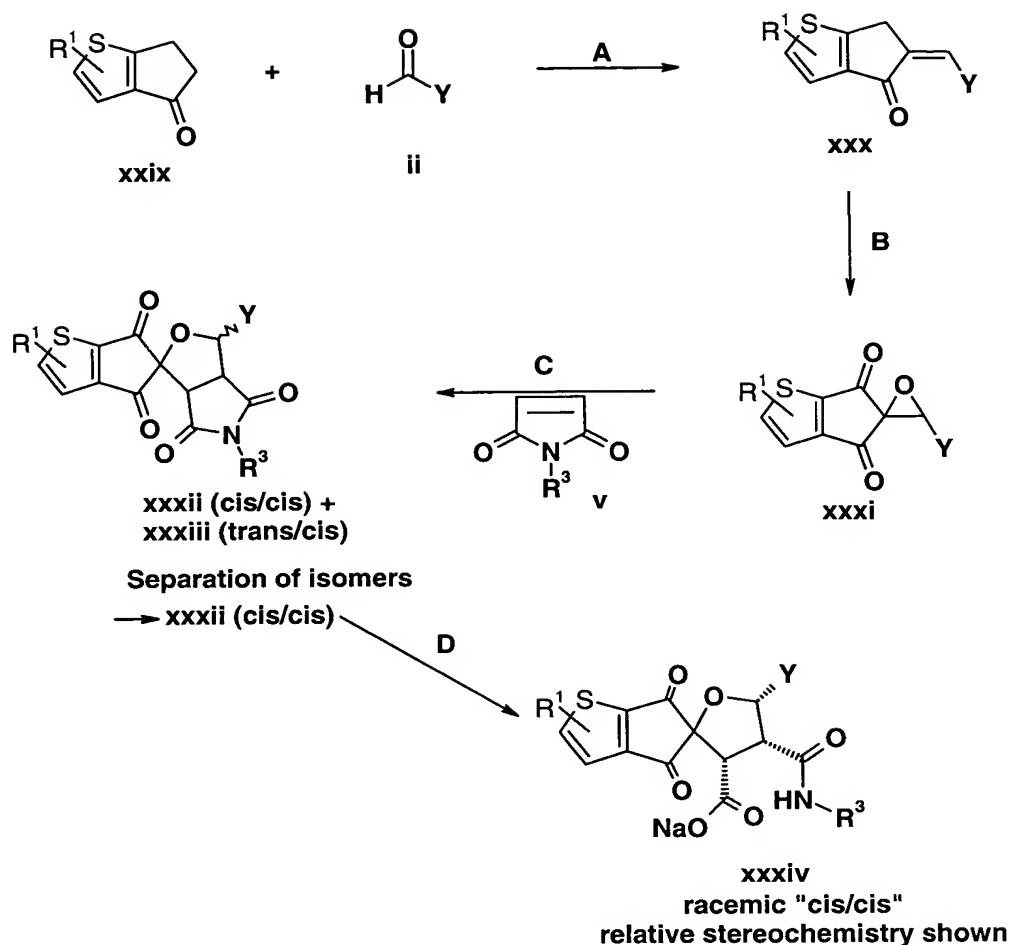
B): Benzylidene (xxiii) is converted to the epoxide (xxiv) by base-catalyzed oxidation with hydrogen peroxide in a protic solvent (such as methanol).

- 5 C): Epoxide (xxiv) undergoes thermal 1,3-dipolar cycloaddition in the presence of maleimide (v) at temperatures ranging from 80 to 100 °C in a solvent such as toluene or xylene (ref.: M.Y. Krysin, I.K. Anohina, L.P. Zalukaev; Khimiya Geterotsiklicheskikh Soedinenii, 1987, 11, 1463-1466). Thus racemic "*cis/cis*" (xxvi) and racemic "*cis/trans*" (xxvii) are obtained after
- 10 purification (crystallization, flash column chromatography, or preparative HPLC). In general maleimides such as (v) are commercially available or alternatively can be easily prepared using literature procedures (e.g. P.Y. Reddy, S. Kondo, T. Toru, Y. Ueno; J. Org. Chem., 1997, 62, 2652-2654).

- 15 D): Racemic "*cis/cis*" compound (xxvi) is hydrolyzed to yield its opened carboxylate form (xxviii) also as "*cis/cis*" racemic mixture. Hydrolysis is achieved under aqueous basic conditions, such as aqueous sodium hydroxide and acetonitrile as a co-solvent.

An alternate route to compounds of the formula I" is shown in Scheme VII.

## Scheme VII



- A) Compound xxix [prepared by homologation of commercially available 5-methyl-2-thiophenecarboxaldehyde with malonic acid, followed by reduction of the exocyclic double bond with sodium amalgam, or hydrogen over palladium, followed by cyclization with oxalyl chloride/ $\text{AlCl}_3$ , or polyphosphoric acid] is condensed with aldehyde (ii) in the presence of a catalytic amount of an acid catalyst (e.g. *p*-toluene sulfonic acid) in benzene or toluene, to form the benzylidene (xxx).  
 B) Benzylidene (xxx) is converted to the epoxide (xxxi) by oxidation (e.g.  $\text{CrO}_3/t$ -butylhydroperoxide)  
 C) Epoxide (xxxi) undergoes thermal 1,3-dipolar cycloaddition in the presence of maleimide (v) at temperatures ranging from 80 to 100 °C in a solvent such as toluene or xylene (ref.: M.Y. Krysin, I.K. Anohina, L.P. Zalukaev;

Khimiya Geterotsiklicheskikh Soedinenii, 1987, 11, 1463-1466). Thus racemic "*cis/cis*" (xxxii) and racemic "*cis/trans*" (xxxiii) are obtained after purification (crystallization, flash column chromatography, or preparative HPLC). In general maleimides such as (v) are commercially available or  
 5 alternatively can be easily prepared using literature procedures (e.g. P.Y. Reddy, S. Kondo, T. Toru, Y. Ueno; J. Org. Chem., 1997, 62, 2652-2654).

10 D) Racemic "*cis/cis*" compound (xxxii) is hydrolyzed to yield its opened carboxylate form (xxxiv) also as "*cis/cis*" racemic mixture. Hydrolysis is achieved under aqueous basic conditions, such as aqueous sodium hydroxide and acetonitrile as a co-solvent.

11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009  
1010  
1011  
1012  
1013  
1014  
1015  
1016  
1017  
1018  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028  
1029  
1030  
1031  
1032  
1033  
1034  
1035  
1036  
1037  
1038  
1039  
1040  
1041  
1042  
1043  
1044  
1045  
1046  
1047  
1048  
1049  
1050  
1051  
1052  
1053  
1054  
1055  
1056  
1057  
1058  
1059  
1060  
1061  
1062  
1063  
1064  
1065  
1066  
1067  
1068  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1080  
1081  
1082  
1083  
1084  
1085  
1086  
1087  
1088  
1089  
1090  
1091  
1092  
1093  
1094  
1095  
1096  
1097  
1098  
1099  
1100  
1101  
1102  
1103  
1104  
1105  
1106  
1107  
1108  
1109  
1110  
1111  
1112  
1113  
1114  
1115  
1116  
1117  
1118  
1119  
1120  
1121  
1122  
1123  
1124  
1125  
1126  
1127  
1128  
1129  
1130  
1131  
1132  
1133  
1134  
1135  
1136  
1137  
1138  
1139  
1140  
1141  
1142  
1143  
1144  
1145  
1146  
1147  
1148  
1149  
1150  
1151  
1152  
1153  
1154  
1155  
1156  
1157  
1158  
1159  
1160  
1161  
1162  
1163  
1164  
1165  
1166  
1167  
1168  
1169  
1170  
1171  
1172  
1173  
1174  
1175  
1176  
1177  
1178  
1179  
1180  
1181  
1182  
1183  
1184  
1185  
1186  
1187  
1188  
1189  
1190  
1191  
1192  
1193  
1194  
1195  
1196  
1197  
1198  
1199  
1200  
1201  
1202  
1203  
1204  
1205  
1206  
1207  
1208  
1209  
1210  
1211  
1212  
1213  
1214  
1215  
1216  
1217  
1218  
1219  
1220  
1221  
1222  
1223  
1224  
1225  
1226  
1227  
1228  
1229  
1230  
1231  
1232  
1233  
1234  
1235  
1236  
1237  
1238  
1239  
1240  
1241  
1242  
1243  
1244  
1245  
1246  
1247  
1248  
1249  
1250  
1251  
1252  
1253  
1254  
1255  
1256  
1257  
1258  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276  
1277  
1278  
1279  
1280  
1281  
1282  
1283  
1284  
1285  
1286  
1287  
1288  
1289  
1290  
1291  
1292  
1293  
1294  
1295  
1296  
1297  
1298  
1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309  
1310  
1311  
1312  
1313  
1314  
1315  
1316  
1317  
1318  
1319  
1320  
1321  
1322  
1323  
1324  
1325  
1326  
1327  
1328  
1329  
1330  
1331  
1332  
1333  
1334  
1335  
1336  
1337  
1338  
1339  
1340  
1341  
1342  
1343  
1344  
1345  
1346  
1347  
1348  
1349  
1350  
1351  
1352  
1353  
1354  
1355  
1356  
1357  
1358  
1359  
1360  
1361  
1362  
1363  
1364  
1365  
1366  
1367  
1368  
1369  
1370  
1371  
1372  
1373  
1374  
1375  
1376  
1377  
1378  
1379  
1380  
1381  
1382  
1383  
1384  
1385  
1386  
1387  
1388  
1389  
1390  
1391  
1392  
1393  
1394  
1395  
1396  
1397  
1398  
1399  
1400  
1401  
1402  
1403  
1404  
1405  
1406  
1407  
1408  
1409  
1410  
1411  
1412  
1413  
1414  
1415  
1416  
1417  
1418  
1419  
1420  
1421  
1422  
1423  
1424  
1425  
1426  
1427  
1428  
1429  
1430  
1431  
1432  
1433  
1434  
1435  
1436  
1437  
1438  
1439  
1440  
1441  
1442  
1443  
1444  
1445  
1446  
1447  
1448  
1449  
1450  
1451  
1452  
1453  
1454  
1455  
1456  
1457  
1458  
1459  
1460  
1461  
1462  
1463  
1464  
1465  
1466  
1467  
1468  
1469  
1470  
1471  
1472  
1473  
1474  
1475  
1476  
1477  
1478  
1479  
1480  
1481  
1482  
1483  
1484  
1485  
1486  
1487  
1488  
1489  
1490  
1491  
1492  
1493  
1494  
1495  
1496  
1497  
1498  
1499  
1500  
1501  
1502  
1503  
1504  
1505  
1506  
1507  
1508  
1509  
1510  
1511  
1512  
1513  
1514  
1515  
1516  
1517  
1518  
1519  
1520  
1521  
1522  
1523  
1524  
1525  
1526  
1527  
1528  
1529  
1530  
1531  
1532  
1533  
1534  
1535  
1536  
1537  
1538  
1539  
1540  
1541  
1542  
1543  
1544  
1545  
1546  
1547  
1548  
1549  
1550  
1551  
1552  
1553  
1554  
1555  
1556  
1557  
1558  
1559  
1560  
1561  
1562  
1563  
1564  
1565  
1566  
1567  
1568  
1569  
1570  
1571  
1572  
1573  
1574  
1575  
1576  
1577  
1578  
1579  
1580  
1581  
1582  
1583  
1584  
1585  
1586  
1587  
1588  
1589  
1590  
1591  
1592  
1593  
1594  
1595  
1596  
1597  
1598  
1599  
1600  
1601  
1602  
1603  
1604  
1605  
1606  
1607  
1608  
1609  
1610  
1611  
1612  
1613  
1614  
1615  
1616  
1617  
1618  
1619  
1620  
1621  
1622  
1623  
1624  
1625  
1626  
1627  
1628  
1629  
1630  
1631  
1632  
1633  
1634  
1635  
1636  
1637  
1638  
1639  
1640  
1641  
1642  
1643  
1644  
1645  
1646  
1647  
1648  
1649  
1650  
1651  
1652  
1653  
1654  
1655  
1656  
1657  
1658  
1659  
1660  
1661  
1662  
1663  
1664  
1665  
1666  
1667  
1668  
1669  
1670  
1671  
1672  
1673  
1674  
1675  
1676  
1677  
1678  
1679  
1680  
1681  
1682  
1683  
1684  
1685  
1686  
1687  
1688  
1689  
1690  
1691  
1692  
1693  
1694  
1695  
1696  
1697  
1698  
1699  
1700  
1701  
1702  
1703  
1704  
1705  
1706  
1707  
1708  
1709  
1710  
1711  
1712  
1713  
1714  
1715  
1716  
1717  
1718  
1719  
1720  
1721  
1722  
1723  
1724  
1725  
1726  
1727  
1728  
1729  
1730  
1731  
1732  
1733  
1734  
1735  
1736  
1737  
1738  
1739  
1740  
1741  
1742  
1743  
1744  
1745  
1746  
1747  
1748  
1749  
1750  
1751  
1752  
1753  
1754  
1755  
1756  
1757  
1758  
1759  
1760  
1761  
1762  
1763  
1764  
1765  
1766  
1767  
1768  
1769  
1770  
1771  
1772  
1773  
1774  
1775  
1776  
1777  
1778  
1779  
1780  
1781  
1782  
1783  
1784  
1785  
1786  
1787  
1788  
1789  
1790  
1791  
1792  
1793  
1794  
1795  
1796  
1797  
1798  
1799  
1800  
1801  
1802  
1803  
1804  
1805  
1806  
1807  
1808  
1809  
1810  
1811  
1812  
1813  
1814  
1815  
1816  
1817  
1818  
1819  
1820  
1821  
1822  
1823  
1824  
1825  
1826  
1827  
1828  
1829  
1830  
1831  
1832  
1833  
1834  
1835  
1836  
1837  
1838  
1839  
1840  
1841  
1842  
1843  
1844  
1845  
1846  
1847  
1848  
1849  
1850  
1851  
1852  
1853  
1854  
1855  
1856  
1857  
1858  
1859  
1860  
1861  
1862  
1863  
1864  
1865  
1866  
1867  
1868  
1869  
1870  
1871  
1872  
1873  
1874  
1875  
1876  
1877  
1878  
1879  
1880  
1881  
1882  
1883  
1884  
1885  
1886  
1887  
1888  
1889  
1890  
1891  
1892  
1893  
1894  
1895  
1896  
1897  
1898  
1899  
1900  
1901  
1902  
1903  
1904  
1905  
1906  
1907  
1908  
1909  
1910  
1911  
1912  
1913  
1914  
1915  
1916  
1917  
1918  
1919  
1920  
1921  
1922  
1923  
1924  
1925  
1926  
1927  
1928  
1929  
1930  
1931  
1932  
1933  
1934  
1935  
1936  
1937  
1938  
1939  
1940  
1941  
1942  
1943  
1944  
1945  
1946  
1947  
1948  
1949  
1950  
1951  
1952  
1953  
1954  
1955  
1956  
1957  
1958  
1959  
1960  
1961  
1962  
1963  
1964  
1965  
1966  
1967  
1968  
1969  
1970  
1971  
1972  
1973  
1974  
1975  
1976  
1977  
1978  
1979  
1980  
1981  
1982  
1983  
1984  
1985  
1986  
1987  
1988  
1989  
1990  
1991  
1992  
1993  
1994  
1995  
1996  
1997  
1998  
1999  
2000  
2001  
2002  
2003  
2004  
2005  
2006  
2007  
2008  
2009  
2010  
2011  
2012  
2013  
2014  
2015  
2016  
2017  
2018  
2019  
2020  
2021  
2022  
2023  
2024  
2025  
2026  
2027  
2028  
2029  
2030  
2031  
2032  
2033  
2034  
2035  
2036  
2037  
2038  
2039  
2040  
2041  
2042  
2043  
2044  
2045  
2046  
2047  
2048  
2049  
2050  
2051  
2052  
2053  
2054  
2055  
2056  
2057  
2058  
2059  
2060  
2061  
2062  
2063  
2064  
2065  
2066  
2067  
2068  
2069  
2070  
2071  
2072  
2073  
2074  
2075  
2076  
2077  
2078  
2079  
2080  
2081  
2082  
2083  
2084  
2085  
2086  
2087  
2088  
2089  
2090  
2091  
2092  
2093  
2094  
2095  
2096  
2097  
2098  
2099  
2100  
2101  
2102  
2103  
2104  
2105  
2106  
2107  
2108  
2109  
2110  
2111  
2112  
2113  
2114  
2115  
2116  
2117  
2118  
2119  
2120  
2121  
2122  
2123  
2124  
2125  
2126  
2127  
2128  
2129  
2130  
2131  
2132  
2133  
2134  
2135  
2136  
2137  
2138  
2139  
2140  
2141  
2142  
2143  
2144  
2145  
2146  
2147  
2148  
2149  
2150  
2151  
2152  
2153  
2154  
2155  
2156  
2157  
2158  
2159  
2160  
2161  
2162  
2163  
2164  
2165  
2166  
2167  
2168  
2169  
2170  
2171  
2172  
2173  
2174  
2175  
2176  
2177  
2178  
2179  
2180  
2181  
2182  
2183  
2184  
2185  
2186  
2187  
2188  
2189  
2190

## EXAMPLES

The present invention is illustrated in further detail by the following non-limiting examples. All reactions were performed in a nitrogen or argon atmosphere.

Temperatures are given in degrees Celsius. Solution percentages or ratios

5 express a volume to volume relationship, unless stated otherwise.

Abbreviations or symbols used herein include:

DEAD: diethyl azodicarboxylate;

DIEA: diisopropylethylamine;

DMAP: 4-(dimethylamino)pyridine;

10 DMSO: dimethylsulfoxide;

DMF: dimethylformamide;

ES MS: electron spray mass spectrometry;

Et: ethyl;

EtOAc: ethyl acetate;

15 Et<sub>2</sub>O: diethyl ether;

HPLC: high performance liquid chromatography;

*i*Pr: isopropyl

Me: methyl;

MeOH: methanol;

20 MeCN: acetonitrile;

Ph: phenyl;

TBE: tris-borate-EDTA;

TBTU: 2-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium  
tetrafluoroborate;

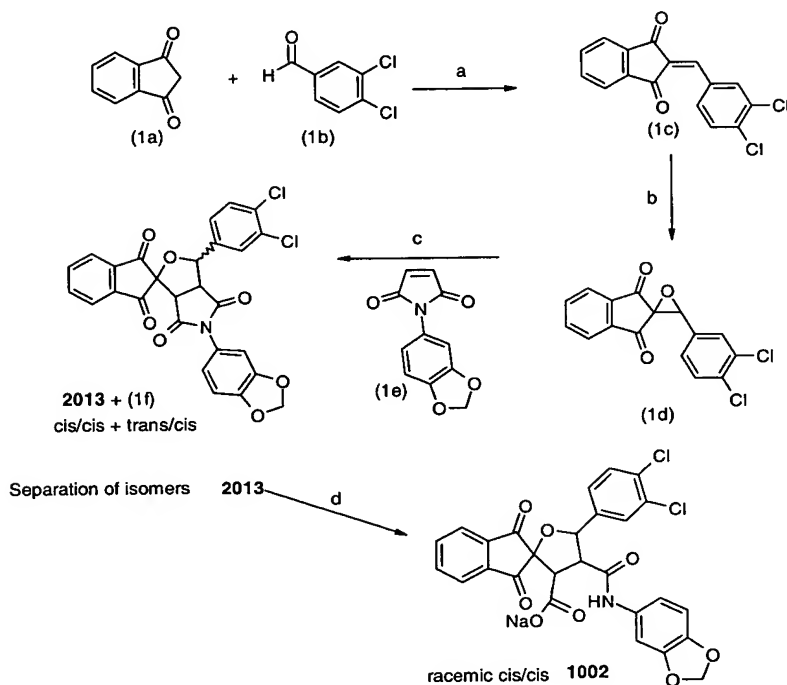
25 TFA: trifluoroacetic acid;

THF: tetrahydrofuran;

MS (FAB) or FAB/MS: fast atom bombardment mass spectrometry;

HRMS: high resolution mass spectrometry;

**EXAMPLE 1: PREPARATION OF COMPOUNDS 2013 (TABLE 2) AND 1002 (TABLE 1)**



5 Step a:

To a solution of indan-1, 3-dione (**1a**) (960 mg, 6.6 mmol) in EtOH (8.2 mL) was added 3, 4-dichlorobenzaldehyde (**1b**) (1.3 g, 7.2 mmol) followed by piperidine (3 drops). The reaction mixture was heated to reflux for 30 min. After cooling, the reaction was diluted with EtOH (8 mL) and the precipitate was filtered. The resulting solid was triturated twice with EtOH and dried under high vacuum to give 2-(3,4-dichloro-benzylidene)-indane-1, 3-dione (**1c**) (1.7 g, 82% yield).

**Step b:**

15 To a suspension of 2-(3,4-dichloro-benzylidene)-indane-1, 3-dione (**1c**) (1.6 g, 5.2 mmol) in MeOH (13 mL) was added hydrogen peroxide (30% solution, 3 mL). The mixture was cooled to 0°C and sodium hydroxide (1N, 300  $\mu$ L) was added dropwise. After addition was completed, stirring was continued at room temperature for 1 h. The mixture was then poured into water (5 mL) and the  
20 resulting solid was collected by filtration and washed with water and MeOH.

After drying under high vacuum 3-(3,4-dichlorophenyl)-spiro (oxirane-2, 2'-indan)-1', 3'-dione (**1d**) (1.6 g, 95 % yield) was obtained.

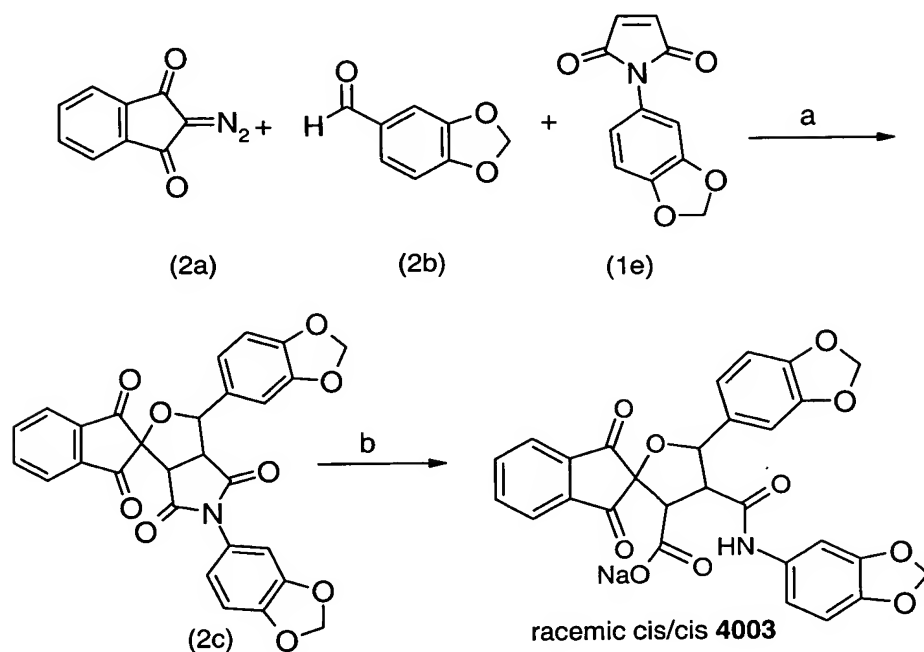
**Step c: compound 2013**

- 5 A mixture of 3-(3,4-dichlorophenyl)-spiro (oxirane-2, 2'-indan)-1', 3'-dione (**1d**) (11 g, 33.4 mmol) and 1-benzo (1,3) dioxol-5-yl-pyrrol-2,5-dione (**1e**) (7.3 g, 33.4 mmol) in toluene (167 mL) was heated to reflux for 16 h. After cooling and concentration, the residue was purified by flash chromatography (SiO<sub>2</sub>, gradient 50% EtOAc/hexane to 30% hexane/EtOAc) to give compound **2013**
- 10 (Table 2) (*cis/cis* isomer, 17.9 g, 50% yield) and (**1f**) (*trans/cis* isomer, 4.1 g, 23% yield).

**Step d: compound 1002**

- To a solution of (**2013**) (143 mg, 0.27 mmol) in CH<sub>3</sub>CN (27 mL) was added
- 15 NaOH (0.02N, 135 mL, 0.27 mmol) using a syringe pump over 1 h. After the addition was completed, the reaction mixture was stirred for an extra 2 h and the resulting solution was concentrated and lyophilized to give compound **1002** (Table 1) (161 mg, 100% yield) as a white solid.

## EXAMPLE 2: PREPARATION OF COMPOUND 4003 (TABLE 4)



## Preparation of compound (2a):

To a suspension of sodium azide (2.4 g, 36.3 mmol) in CH<sub>3</sub>CN (73 mL) was added methanesulfonyl chloride (2.8 mL, 36.3 mmol). After stirring for 16 h, the reaction mixture was poured over a suspension of indan-1, 3-dione (5.3 g, 36.3 mmol) and cesium carbonate (11.8 g, 36.3 mmol) in CH<sub>3</sub>CN (50 mL).

The mixture was stirred until reaction was complete (2 h), and then filtered through Celite. The filter cake was washed with EtOAc and the organic solvents were removed *in vacuo*. The resulting gum was diluted with EtOAc and rinsed successively with NaOH (1N), H<sub>2</sub>O and brine. The organic layer was dried (MgSO<sub>4</sub>), evaporated and the crude oil purified by flash chromatography (SiO<sub>2</sub>, 50% hexane/EtOAc) to give 2-diazoindan-1,3-dione (2a) (2.6 g, 42% yield).

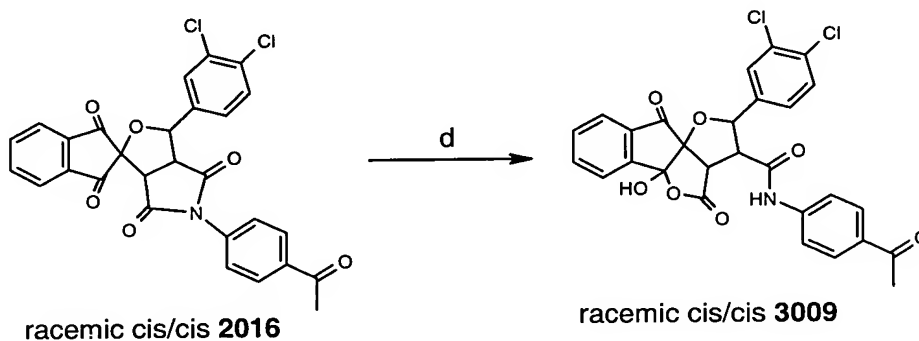
Step a:

To a mixture of diazoindan-1, 3-dione (**2a**) (317 mg, 1.8 mmol), piperonal (**2b**) (553 mg, 3.7 mmol) and 1-benzo (1,3) dioxol-5-yl-pyrrol-2,5-dione (**1e**) (400 mg, 1.8 mmol) in benzene (6 mL) and  $\text{Rh}_2(\text{OAc})_4$  (4.5 mg) were added and  
 5 heated to reflux for 1 h. After cooling and concentrating, the reaction mixture was purified by flash chromatography ( $\text{SiO}_2$ , gradient 50% hexane/EtOAc to 30% hexane/EtOAc) to give the desired compound (**2c**) as beige solid (76.5 mg, 8% yield).

10 Step b: compound **4003**

Following the same procedure as in Example 1, step d, compound **4003** was obtained as a white solid.

### EXAMPLE 3: PREPARATION OF COMPOUND 3009 (TABLE 3)



15

Compound **2016** was prepared as described in Example 1, steps a to c using N-(4-acetylphenyl)maleimide in the cycloaddition (step c) to give a white solid in 40 % yield.

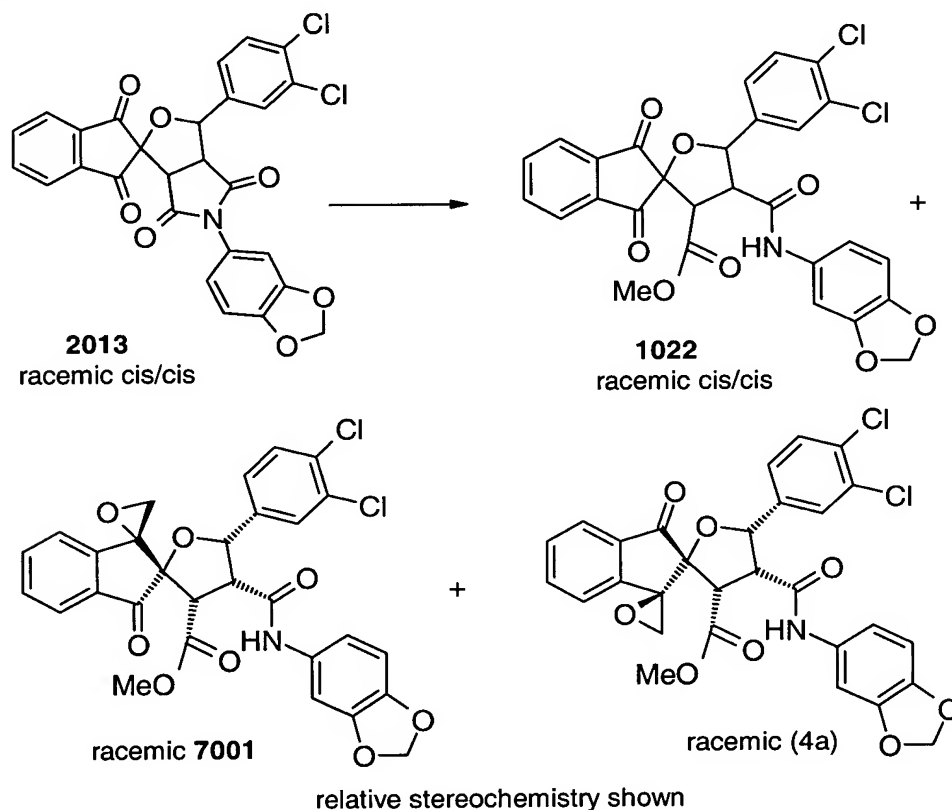
20

step d:

To a solution of **2016** (100 mg, 0.19 mmol) in  $\text{CH}_3\text{CN}$  was added NaOH (0.02 N, 9.4 mL, 0.19 mmol) over 1 h using a syringe pump. After addition was complete, the solution was concentrated and lyophilized to give a white solid  
 25 which was purified on reverse phase HPLC using a gradient of  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$

containing TFA (0.06%). After collecting the desired fractions and lyophilization **compound 3009** was obtained (43 mg, 42% yield) as a white solid.

5 **EXAMPLE 4: PREPARATION OF COMPOUND 1022 (TABLE 1) AND COMPOUND 7001 (TABLE 7)**

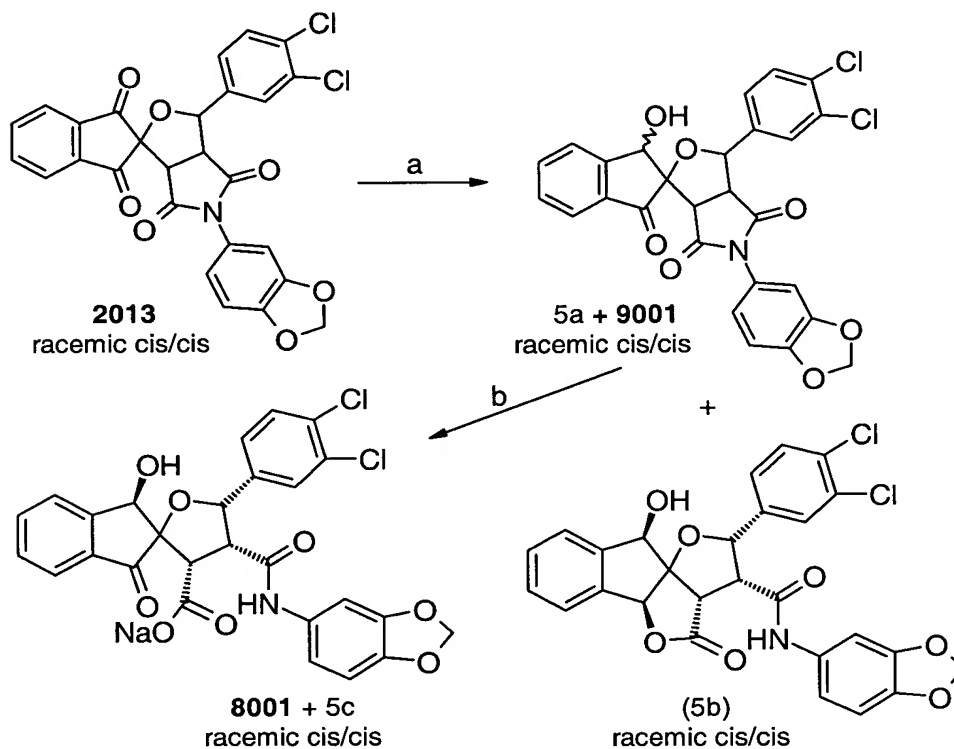


To a solution of **2013** (racemic) (143 mg, 0.27 mmol) in CH<sub>3</sub>CN (27 mL) was added NaOH (0.02N, 135 mL, 0.27 mmol) using a syringe pump over 1 h.

- 10 After addition was complete, the reaction mixture was concentrated. To the aqueous layer was added HCl (1N, 1 mL) and the resulting acidic layer was extracted twice with EtOAc. The combined organic layer was washed with H<sub>2</sub>O and brine, dried (MgSO<sub>4</sub>) and filtered. To the filtrate was added a solution of CH<sub>2</sub>N<sub>2</sub> in Et<sub>2</sub>O (excess) and the mixture was evaporated to
- 15 dryness. The resulting crude compound was purified by preparative HPLC using a gradient of CH<sub>3</sub>CN/H<sub>2</sub>O containing TFA (0.06%). After collecting and lyophilizing the desired fractions, compounds **1022** (11 mg, 12% yield), (**4a**) (9

mg, 10% yield), and **7001** (47 mg, 26% yield) as racemic mixtures were obtained as white solids.

#### EXAMPLE 5: PREPARATION OF COMPOUND 8001



relative stereochemistry shown

#### Step a:

To a solution of **2013** (racemic) (500 mg, 0.93 mmol) in THF/MeOH mixture (20/5 mL) cooled at 0°C was added NaBH<sub>4</sub> (35 mg, 0.93 mmol). After stirring for 30 min, the reaction was quenched with aqueous citric acid solution (10%) and diluted with EtOAc. The aqueous layer was extracted twice with EtOAc and the combined organic layers were washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated. Preparative HPLC gave racemic alcohols (**5a**) + **9001** (360 mg, 72% yield, retention times: 14.1 and 12.4 min) as well as hydroxylactone (**5b**) (95 mg, 18% yield, retention time: 13.8 min).

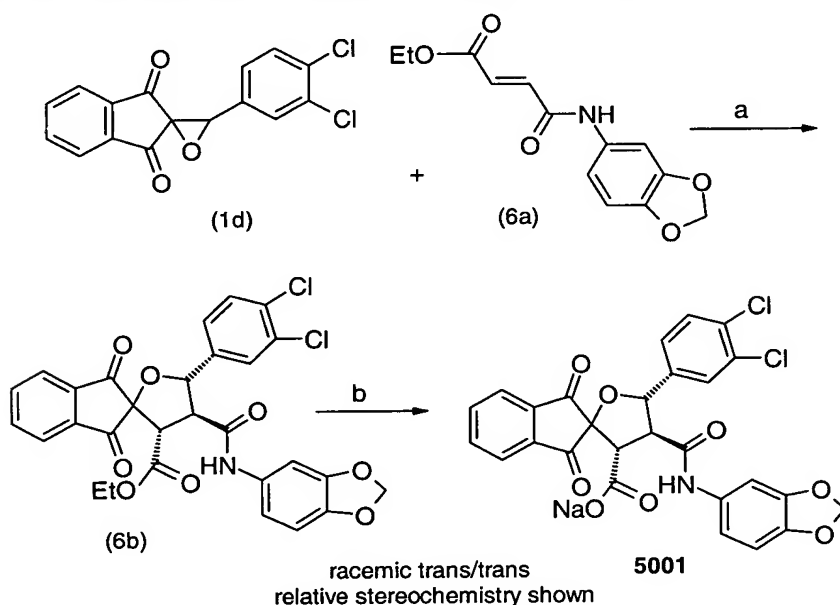
The mixture of alcohols was separated by preparative HPLC to generate the

two isomeric compounds as white solids:

Step b:

To the mixture of alcohols (**5a**) + **9001** (2:1 ratio, 36 mg, 0.07 mmol) in CH<sub>3</sub>CN (10 mL) was added NaOH (0.02N, 3.4 mL, 0.07 mmol) over 2 h using a syringe pump. The CH<sub>3</sub>CN was evaporated and the residue was purified by preparative HPLC. The desired fractions were lyophilized and the resulting solids were retreated with NaOH (1 equiv.) and lyophilized to give compound **8001** (21.3 mg, 59% yield, retention time: 13.7 min) and (**5c**) (6.0 mg, 17% yield, retention time: 10.9 min) as white solids.

#### EXAMPLE 6: PREPARATION OF COMPOUND 5001 (TABLE 5)



Preparation of amide (**6a**):

A mixture of monoethyl fumarate (1 g, 6.9 mmol) and hexachloroacetone (0.5 mL, 3.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (13 mL) was stirred under nitrogen and cooled to –78 °C. Triphenylphosphine (1.8 g, 6.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6.9 mL) was added dropwise and the mixture was stirred for 20 min. The acyl chloride solution was then treated with a solution of 3,4-methylenedioxyaniline (946 mg, 6.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6.9 mL) followed by Et<sub>3</sub>N (0.96 mL, 6.9 mmol) in CH<sub>2</sub>Cl<sub>2</sub>

(6.9 mL). The reaction mixture was allowed to warm to room temperature after which the solvent was removed under high vacuum. The resulting residue was purified by flash chromatography (SiO<sub>2</sub>, 20% EtOAc/hexane to give the desired amide (**6a**) (961 mg, 53% yield) as an orange solid.

5

Step a:

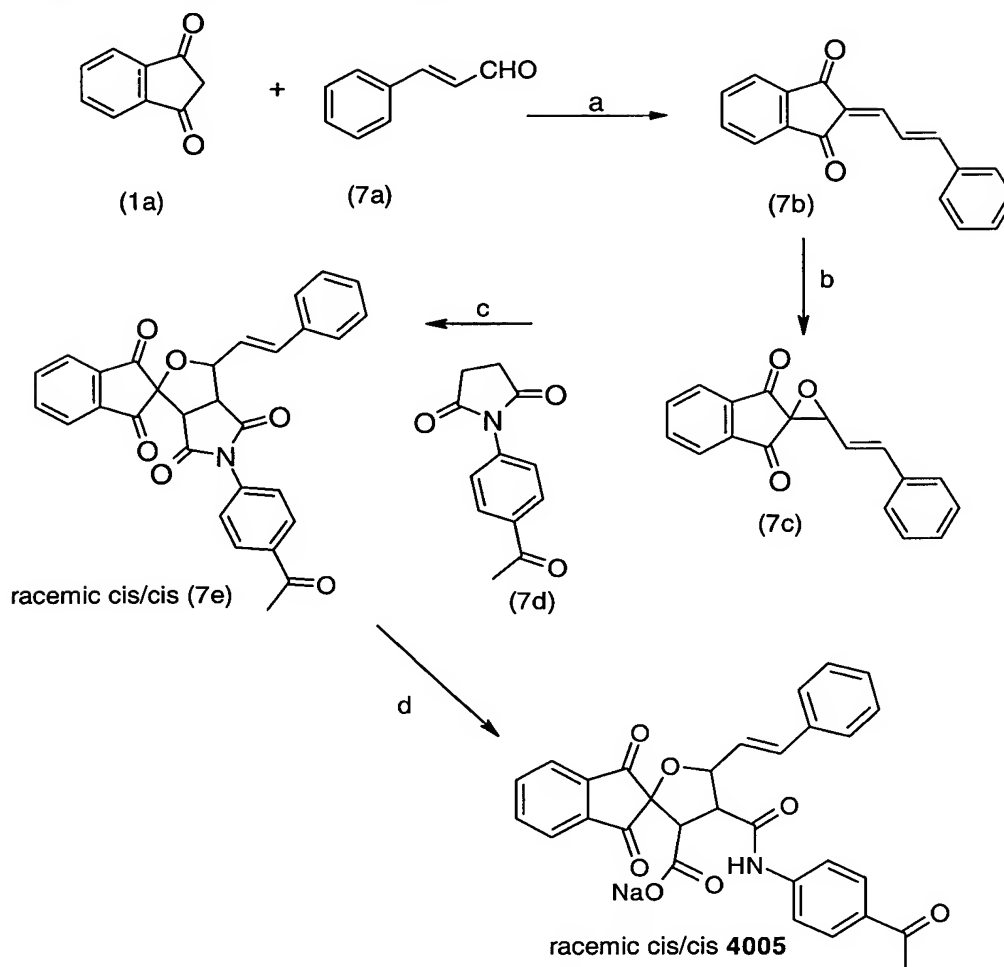
Amide (**6a**) was reacted with epoxide (**1d**) using the same procedure as in Example 1, step c to give ester (**6b**) (77 mg, 14% yield) as an orange solid.

10

Step b:

Using the same procedure as in Example 1, step d, compound **5001** was obtained as a white solid (8 mg, 25 % yield).

1000 800 600 400 200 0

**EXAMPLE 7: PREPARATION OF COMPOUND 4005 (TABLE 4)**step a:

The same procedure as in Example 1, step a, was followed but using indan-  
 5 1,3-dione and trans-cinnamaldehyde (7a) as starting material, to give  
 compound (7b) after purification (11% yield) as an orange solid.

steps b & c:

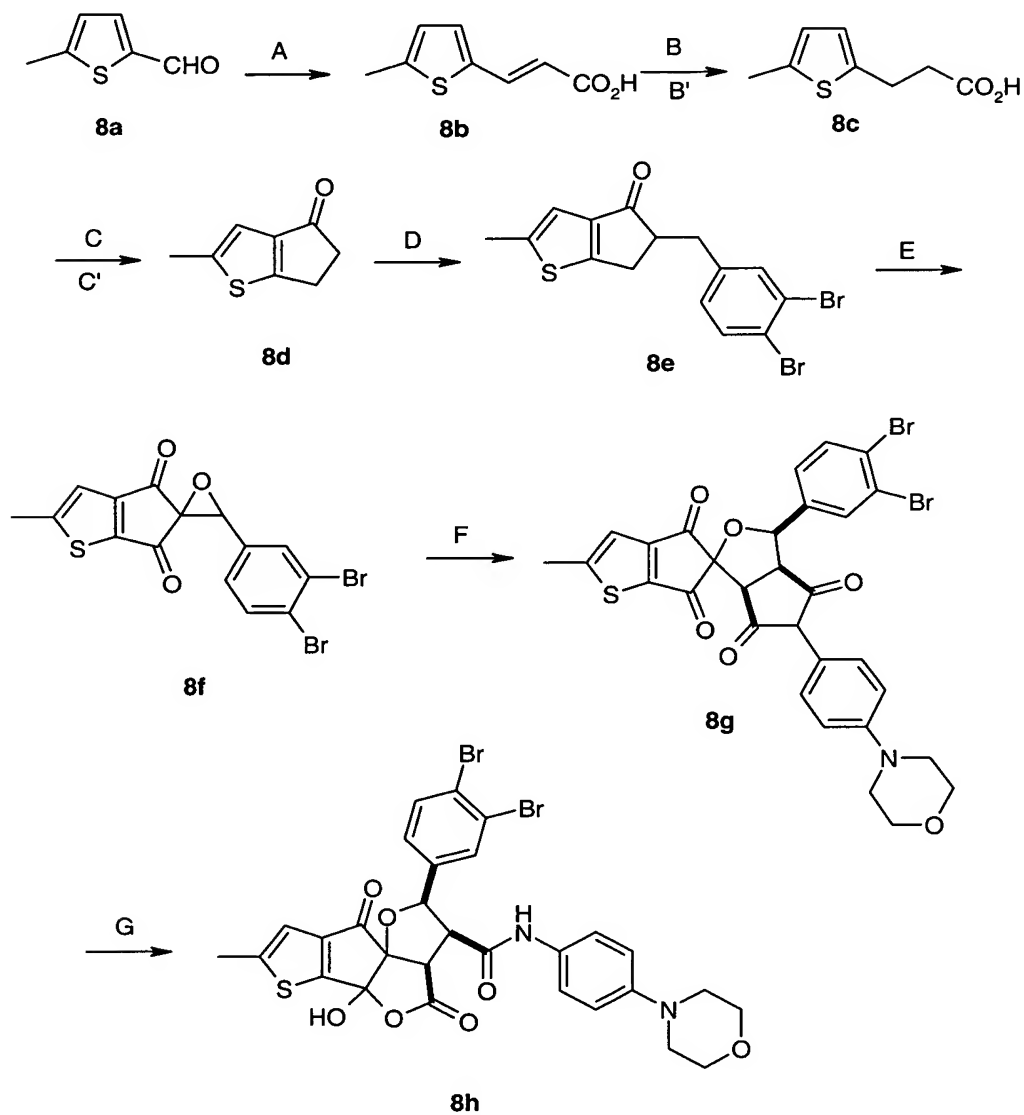
The same procedures as in Example 1, steps b and c were used but using N-  
 10 (n-acetylphenyl)maleimide (7d) in the cycloaddition (step c) to afford  
 compound (7e) (38% yield) as a white solid.

step d:

Hydrolysis was achieved as described in Example 1, step d, to give compound **4005** (racemic) (50% yield) as a white solid.

**EXAMPLE 8: PREPARATION OF COMPOUND # D1002 (TABLE 1D)**

5



**A:** A solution of **8a** (9.5 g, 75.4 mmol), malonic acid (15.7 g, 151 mmol) and piperidine (1.3 mL) in pyridine (40 mL) was refluxed overnight. The resulting mixture was allowed to cool to room temperature whereupon water (200 mL) was added. The mixture was acidified by the addition of concentrated HCl and

10

allowed to stir for 1 h. The mixture was filtered and the solid washed with water. Drying under vacuum gave **8b** as a yellow powder (12.8 g, 100 %).

**B:** To a vigorously stirred solution of **8b** (5.9 g, 35 mmol) and 1 N NaOH (46 mL, 46 mmol) in water (40 mL) was added 2% sodium amalgam (82 g, 105 mmol) in small portions over 1h. After complete addition the mixture was stirred for a further hour. Mercury was removed by decanting and the aqueous solution was acidified with concentrated HCl. Solid NaCl was added to saturation and the resulting mixture was extracted with ether. The combined etherial extracts were washed with brine and dried over MgSO<sub>4</sub>. Removal of solvent under reduced pressure gave **8c** as a brown solid (3.72 g, 62 %).

**B:** Alternatively, a slurry of **8b** (7.5 g, 44.6 mmol) and Pd(OH)<sub>2</sub> (500 mg) in ethanol was stirred under an atmosphere of hydrogen for 18 h. Filtering through glass microfibre and removal of solvent gave **3** as a white solid (7.0 g, 93 %).

**C:** To a solution of **8c** (1.75 g, 10.3 mmol) and oxalyl chloride (1.35 mL, 15.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added one drop of DMF. The resulting solution was stirred at room temperature for 2h. The solvent was then removed under reduced pressure and the resulting residue dissolved in CS<sub>2</sub> (50 mL). Solid AlCl<sub>3</sub> (2.05 g, 15.4 mmol) was then introduced and the resulting mixture refluxed overnight. Ice (80 g) was then added followed by concentrated HCl (30 mL) and the resulting mixture was stirred for 30 min. Extraction with CH<sub>2</sub>Cl<sub>2</sub> was followed by washing with 1N NaOH, brine and drying (MgSO<sub>4</sub>). Flash chromatography (20 % EtOAc in hexanes) gave **8d** (272 mg, 17 %) as a yellow solid.

**C':** Alternatively, solid **8c** (1.0 g, 5.88 mmol) was added in small portions to warm (75 °C) polyphosphoric acid (8.5 g). Heating was continued at 75 °C for one hour after the addition was complete. Cooling to room temperature was followed by dilution with water and extraction with CH<sub>2</sub>Cl<sub>2</sub> (3 X). The combined organics were dried over MgSO<sub>4</sub> and concentrated. Flash chromatography ( 50 % EtOAc in hexanes) gave **8d** as a white solid (0.31 g, 35 %).

**D:** A solution of **8d** (1.06 g, 6.97 mmol), 3,4-dibromobenzaldehyde (1.84 g,

6.97 mmol) and p-toluenesulfonic acid (100 mg) in benzene (25 mL) was refluxed for 24 h with azeotropic removal of water. Upon cooling and addition of ether (25 mL) a solid precipitated which was filtered to give **8e** as a tan solid (1.35 g, 49 %).

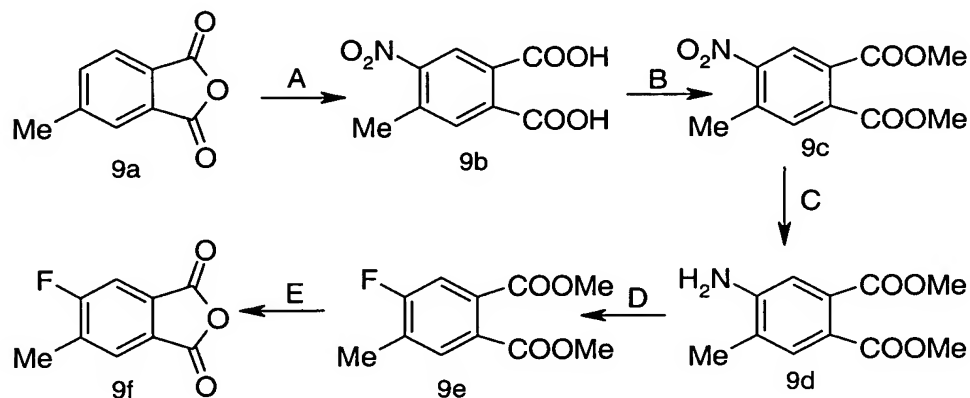
- 5 **E:** To a solution of CrO<sub>3</sub> (50 mg, 0.50 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added *tert*-butylhydroperoxide (2.6 mL of a 70 % solution in water). After stirring for 2 minutes, **8e** (1.0 g, 2.51 mmol) was added. After stirring for 18 h at room temperature the solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> and water and extracted three times with small portions of CH<sub>2</sub>Cl<sub>2</sub>. The combined organics were dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Trituration of the resulting solid with ether gave 0.61 g (60 %) of a solid diketone.
- 10

The material so obtained (0.45 g) was dissolved in EtOH (15 mL) to which was added 30 % H<sub>2</sub>O<sub>2</sub> (0.38 mL) and one drop of 1N NaOH. After stirring for 3 h the solution was filtered to give **8f** as a yellow solid (421 mg, 90 %).

- 15 **F:** A solution of **8f** (0.30 g, 0.70 mmol) and N-[4-(N'-morpholino)phenyl]maleimide (0.18 g, 0.70 mmol) in toluene (8 mL) was refluxed for 48 h. After cooling to room temperature a solid formed was filtered and then triturated with THF to provide **8g** (75 mg, 16 %). **G:** To a solution of **8g** (69 mg, 0.10 mmol) in 40 % THF:CH<sub>3</sub>CN (8 mL) was added NaOH (5.3 mL
- 20 of a 0.02 M solution in water) over 10 h via syringe pump. The reaction mixture was purified directly by preparative HPLC (Chiralcel OD column, isocratic 50 % CH<sub>3</sub>CN/H<sub>2</sub>O, 0.06 % TFA) which afforded enantiomerically pure **8h** (2.5 mg, 4%).

**EXAMPLE 9: PREPARATION OF 5-METHYL-6-FLUORO-1,3-INDANEDIONE**

This material is useful as a starting material for preparation of compounds of the formula I' in which R<sup>1</sup> is c-F and b-Me.



a) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>; b) (MeO)<sub>2</sub>SO<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, DMF; c) H<sub>2</sub>, Pd(OH)<sub>2</sub>/C;  
d) NaNO<sub>2</sub>, HF<sub>x</sub>.Pyr; NaH, EtOAc, then H<sub>3</sub>O<sup>+</sup>

5

**A, B:** 4-Methylphthalic anhydride **9a** (67.5 mmol, 10.94 g) and concentrated sulfuric acid (10 mL) were placed in a three-necked round-bottomed flask and the mixture was stirred mechanically at 80°C. A mixture of fuming nitric acid (d = 1.5, 4.2 mL) and concentrated sulfuric acid (3.0 mL) was added slowly from a dropping funnel at such a rate as to maintain the temperature of the stirred mixture at 100-110°C. Then concentrated nitric acid (d = 1.42, 18 mL) was added as rapidly as possible without causing the temperature to rise above 110°C. The reaction mixture was heated at 100°C for two hours, allowed to stand at room temperature for 16 h and poured into 30 mL of water. The white precipitate was filtered off and the filtrate was extracted with ethyl ether. The organic phase was dried with magnesium sulfate, filtered and concentrated under vacuum. The residual solid (13 g) was dissolved in N,N-dimethylformamide (50 mL) containing potassium carbonate (0.12 mol, 16.9 g). Dimethyl sulfate (0.12 mol, 15.4 g, 11.5 mL) was added and the mixture was stirred magnetically at room temperature for two hours. N,N-dimethylformamide was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the organic phase was washed with water, brine and dried with magnesium sulfate. The salts were filtered off and the filtrate

10

15

20

was concentrated under vacuum. The residue was purified by flash chromatography on type H silica gel using hexane/ethyl acetate (4/1) as eluent to yield 5.26 g (31%) of dimethyl 4-methyl-5-nitrophthalate **9c** as a white solid.

5     **C:** Dimethyl 4-methyl-5-nitrophthalate **9c** (3.41 g, 13.5 mmol) was dissolved in a mixture of methanol (120 mL) and tetrahydrofuran (20 mL). Palladium hydroxide on carbon (20%, 300 mg) was added and the suspension was stirred magnetically at room temperature under hydrogen atmosphere (1 atm) for 16 hours. The reaction mixture was filtered on Celite and the filtrate was  
10 concentrated under vacuum. The residual oil was purified by flash chromatography on type H silica gel using hexane/ethyl acetate (2/1) as eluent, followed by hexane/ethyl acetate (1/1), to give 2.88 g (96%) of a colorless oil which corresponded to dimethyl 4-methyl-5-aminophthalate **9d**.

15     **D:** A teflon reactor was charged with dimethyl 4-methyl-5-aminophthalate **9d** (4.53 g, 20.3 mmol). HF·pyridine (50 mL, *ca* 1.7 mol HF) was added. The reaction mixture was stirred for 5 min at 0°C and sodium nitrite (1.55 g, 22.5 mmol) was added to produce a purple solution. The mixture was stirred for 15 min at room temperature and for 30 min at 120°C. The reaction mixture was poured onto ice and 4 N sodium hydroxide. Ethyl acetate was added and the  
20 mixture was filtered. The organic phase was dried with magnesium sulfate, filtered, washed with 1 M aq HCl, dried again with magnesium sulfate, filtered and concentrated under vacuum. The residual red liquid was purified by flash chromatography on type H silica gel using hexane/ethyl acetate (4/1) as eluent to give 1.54 g (33%) of dimethyl 4-methyl-5-fluorophthalate **9e** as a white solid.

25     **E:** Dimethyl 4-methyl-5-fluorophthalate **9e** (1.65 g, 7.29 mmol) was dissolved in anhydrous ethyl acetate (4 mL). Sodium hydride (348 mg, 14.5 mmol) was added and the mixture was heated at 100°C for 4 hours. The reaction mixture was cooled to room temperature and a mixture containing 10 mL of hexane  
30 and 6 mL of ethyl ether/ethanol (1/1) was added. The yellow preprecipitate was triturated for 5 min, filtered and dried under vacuum. The yellow solid (1.07 g) was then suspended in a solution containing water (22 mL) and concentrated hydrochloric acid (2.2 mL) and the suspension was heated for 17 min at 80°C. The mixture was then lyophilized to give 810 mg (62%) of 5-methyl-6-fluoro-

1,3-indandione **9f** as a beige solid.

Examples of compounds made using 5-methyl-6-fluoro-1,3-indandione are compounds # A1015 and A1016 (Table 1A).

**EXAMPLE 10: SEPARATION OF MIXTURE TO YIELD PURE ENANTIOMERS A1006, A1007 AND A1008 (TABLE 1A)**

Using the same procedure as in Example 1 steps a to d; but starting with 5-methyl indan-1, 3-dione in step a, and using 1-(4-morpholin-4yl-phenyl)-pyrrole-2,5-dione in step c, a mixture of compounds was obtained which was separated on preparative HPLC using a chiral column (Chiracel OD, isocratic eluent 65% CH<sub>3</sub>CN / H<sub>2</sub>O containing 0.06% TFA; UV lamp at 205 nm; flow 7 mL/min.). The resulting three fractions were lyophilized and treated with NaOH (0.02N, 1 equiv.) to give the corresponding sodium salts as white solids.

Compound **A1006** was isolated as a mixture of isomers in a 1:1 ratio.

Compounds **A1007** and **A1008** were each isolated as pure enantiomers.

**EXAMPLE 11: E2-dependent E1 DNA binding assay**

This assay was modeled on a similar assay for SV40 T Antigen described by McKay (J. Mol. Biol., 1981,145:471). A 400bp radiolabeled DNA probe, containing the HPV-11 origin of replication (Chiang *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:5799) was produced by pcr, using plasmid pBluescript™ SK encoding the origin (nucleotides 7886-61 of the HPV-11 genome in unique BAMH1 site) as template and primers flanking the origin. Radiolabel was incorporated as [<sup>33</sup>P]dCTP. Binding assay buffer consisted of: 20 mM Tris pH 7.6, 100 mM NaCl, 1 mM DTT, 1 mM EDTA.

Other reagents used were protein A-SPA beads (type II, Amersham) and K72 rabbit polyclonal antiserum, raised against a peptide corresponding to the C-terminal 14 amino acids of HPV-11 E1. Following the protocol from Amersham, one bottle of beads was mixed with 25 mL of binding assay buffer. For the assay, a saturating amount of K72 antiserum was added to the beads and the mixture was incubated for 1 h, washed with one volume of binding

assay buffer, and then resuspended in the same volume of fresh binding assay buffer. Binding reactions contained 8 ng of E2, approximately 100-200 ng of purified E1, and 0.4 ng of radiolabeled probe in a total of 80  $\mu$ L of binding assay buffer. After 1 h at room temperature, 25  $\mu$ L of K72 antibody-SPA bead suspension was added to with the binding reaction and mixed. After an additional hour of incubation at room temperature, the reactions were centrifuged briefly to pellet the beads and the extent of complex formation was determined by scintillation counting on a Packard TopCount™. Typically, the signal for reactions containing E1 and E2 was 20-30 fold higher than the background observed when either E1, E2, or both was omitted.

#### EXAMPLE 12: SV40 T ANTIGEN-DNA BINDING ASSAY

Selectivity of the inhibitors according to the invention was assessed by activity in the E1 or E1-E2-ori binding assays and lack of activity (or lower potency) in the SV40 large T antigen assay.

This assay measures the formation of an SV40 T Antigen (TAg)-origin complex. The assay was developed by R. D. G. McKay (J. Mol. Biol. (1981) 145, 471-488). In principle, it is very similar to the E2-dependent E1-DNA binding assay (Example 12), with TAg replacing E1 and E2, and a radiolabeled SV40 ori probe replacing the HPV ori probe. The assay is used as a counterscreen for the assay of Example 13, since TAg shares functional homology to E1 and E2, but has very low sequence similarity.

The radiolabeled origin-containing DNA probe was made by PCR using pCH110 plasmid (Pharmacia) as a template. This template encodes the SV40 minimal origin of replication at nucleotides 7098-7023. Primers were "sv40-6958sens" = 5'-GCC CCT AAC TCC GCC CAT CCC GC (**SEQ ID NO. 1**), and "sv40-206anti" = 5'-ACC AGA CCG CCA CGG CTT ACG GC (**SEQ ID NO. 2**). The PCR product was approximately 370 base pairs long and was radiolabeled using 50  $\mu$ Ci/100  $\mu$ L PCR reaction of dCTP ( $\alpha$ -<sup>33</sup>P). Subsequent to the PCR reaction, the product was purified using either the Qiagen® PCR purification kit, or a phenol extraction/ethanol precipitation procedure. The

purified product was diluted to 1.5 ng/ $\mu$ L (estimated by gel electrophoresis) in TE. Fresh preparations had approximately 150,000 cpm/ $\mu$ L.

5 Binding reactions were performed by mixing 30  $\mu$ L of TAg solution (100 ng/well, 200 ng of a  $^{33}$ P-radiolabeled DNA probe, and 7.5  $\mu$ L of 10 x DNA binding buffer (200 mM Tris-HCl pH 7.6, 100 mM NaCl, 1 mM EDTA, 10 mM DTT) in a final volume of 75  $\mu$ L. Binding reactions were allowed to proceed at room temperature for 60 min. The Large T Antigen purchased from Chimerx, at 2.0 mg/mL.

10

The protein-DNA complexes were immunocaptured using an  $\alpha$ -TAg monoclonal antibody (PAb 101, subtype IgG2a, hybridoma obtained from ATCC and antibody purified in-house) bound to protein A-SPA beads. Immunoprecipitation of protein-DNA complexes was carried out for 1 hr at rt.

15 The plates were spun briefly and the precipitated radiolabeled DNA fragments were counted on a TopCount® counter.

### EXAMPLE 13: CELL-BASED DNA REPLICATION ASSAY

CHO-K1 cells were transfected using Lipofectamine Plus Reagent (Gibco/BRL) following standard procedure. Cells grown to 40-60% confluence in 100mm tissue culture dishes were transfected simultaneously with 0.5  $\mu$ g pN9-ORI (HPV-11 minimal origin of DNA replication), 0.5  $\mu$ g pCR3-E1 and 0.05  $\mu$ g pCR3-E2 (containing respectively HPV-11 E1 full length and HPV-11 E2 full length cloned by the TA cloning system). After 4 hours of incubation

20 with the DNA mixture, cells were trypsinized, pooled and replated at 20,000 cells/well in a 96 well plate. Following 2 hours of attachment at 37°C, serially diluted inhibitor compounds were added for a 2 days incubation period.

25 The cells were washed to eliminate the compound, and the total DNA was extracted using a modified protocol of the QIAamp Blood Kit (QIAGEN). DNA was digested with Hind III (10U/well) and Dpn1 (20U/well) for 4 hours at 37°C.

30

Digested DNA (10  $\mu$ l) was subjected to 23 rounds of PCR amplification using the Pwo DNA polymerase Kit (Boehringer Mannheim) modified to contain 2 U of Pwo DNA polymerase, 10  $\mu$ Ci [ $\alpha$ - $^{33}$ P]dCTP and 2 primers (each at a final concentration of 0.2  $\mu$ M) per 50  $\mu$ l reaction.

Cycling consisted of an initial denaturation step at 95°C for 5 min., followed by 23 rounds of: denaturation at 94°C for 30 sec., annealing and extension at 72°C for 1 min. 30 sec., ending with a final extension at 72°C for 10 min. After amplification was completed, 10  $\mu$ l was analyzed on 1% agarose gel, subsequently dried at 60°C for 1 hour, and analyzed by PhosphorImager.

To evaluate the effect of the compound on cellular DNA synthesis (and/or cellular toxicity), cell proliferation ELISA (Boehringer Mannheim), which monitor BrdU incorporation, were performed.

#### EXAMPLE 14: TABLES OF COMPOUNDS

All compounds listed in Tables 1 to 10 were found to be active in the E1-E2 DNA assay presented in Example 11 with an IC<sub>50</sub> under 50 $\mu$ M for HPV-11.

Table legend: For IC<sub>50</sub> A=50 $\mu$ M-5 $\mu$ M; B=5 $\mu$ M-0.5 $\mu$ M; C=<0.5 $\mu$ M

Certain compounds were also tested in the SV40 TAg assay of Example 12 and were found to be inactive or less active than in the E1-E2 DNA assay, providing good evidence that these compounds are selective against the papilloma virus.

In addition, certain compounds were tested in the DNA replication cellular assay of Example 13. The results obtained indicate that they can inhibit viral replication.

Table 1

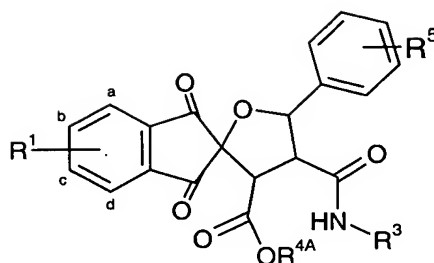
racemic, "*cis/cis*" isomer (form 1)

Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1001	Na	--	4-Cl		520	A
1002	Na	--	3,4-Cl		552*	C
1003	Na	--	4-Cl		492*	A
1004	Na	--	4-Cl		534*	A
1005	Na	--	4-Cl		516*	A
1006	Na	--	4-Cl		508*	A
1007	Na	--	4-Cl		488*	A
1008	Na	--	4- <i>i</i> Pr		526*	A
1009	Na	--	4-Cl		510*	A
1010	Na	--	4-Cl		510*	A

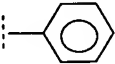
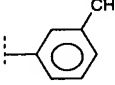
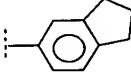
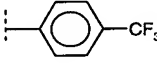
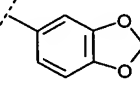
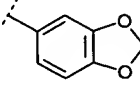
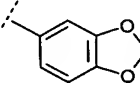
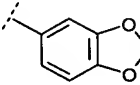
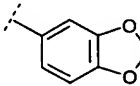
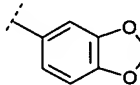
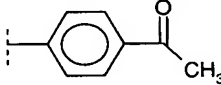
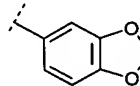
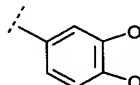
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1011	Na	--	4-Cl		474*	A
1012	Na	--	4-Cl		488*	A
1013	Na	--	4-Cl		514*	A
1014	Na	--	4-Cl		542*	A
1015	Na	--	3-Cl		518*	B
1016	Na	--	4-CF <sub>3</sub>		552*	A
1017	CH <sub>3</sub>	--	4-Cl		532*	A
1018	Na	--	3-CH <sub>3</sub>		498*	A
1019	Na	<i>o</i> -F	4-Cl		536*	A
1020	Na	--	3,5-Cl		552*	A
1021	Na	--	3,4-Cl		552	C
1022	CH <sub>3</sub>	--	3,4-Cl		568	B
1023	Na	--	3-OCH <sub>3</sub>		515*	A

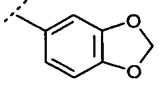
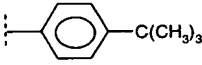
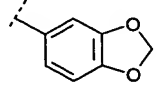
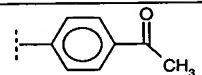
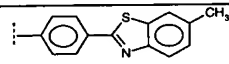
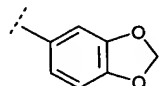
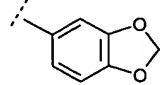
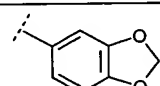
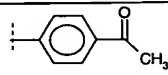
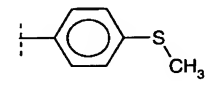
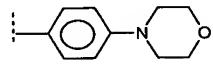
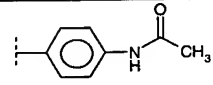
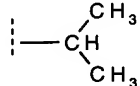
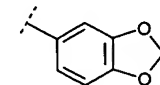
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1024	Na	--	3,4-CH <sub>3</sub>		514	B
1025	Na	--	3,4-Cl		566	B
1026	Na	--	3,4-F		556	A
1027	Na	--	3,4-Br		639*	C
1028	Na	--	3,4-Cl		657	C
1029	Na	--	3-F, 4-Cl		538	B
1030	Na	--	3-Cl, 4-F		538	B
1031	Na	--	3-CF <sub>3</sub>		554	B
1032	Na	--	3-Cl		518	B
1033	Na	--	3,4-Cl		556	B
1034	Na	--	3,4-Cl		595	C
1035	Na	--	3,4-Cl		567	C
1036	Na	--	3,4-Cl		476	B
1037	Na	<i>b</i> -CH <sub>3</sub>	3,4-Cl		568	B

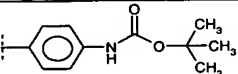
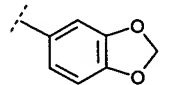
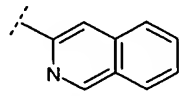
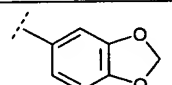
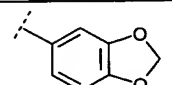
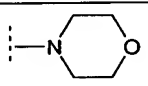
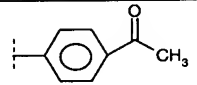
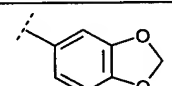
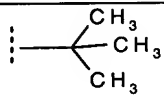
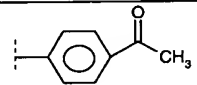
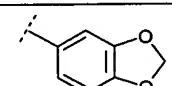
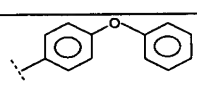
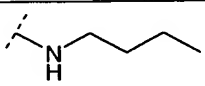
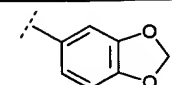
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1038	Na	--	3,4-Cl		525	B
1039	Na	--	4-I		612	B
1040	Na	--	3,4-Cl		561	B
1041	Na	α-CH <sub>3</sub>	3,4-Cl		568	B
1042	Na	α-CH <sub>3</sub>	3,4-Cl		568	B
1043	Na	--	3,4-Cl		519	B
1044	Na	--	3-Cl		519	A
1045	Na	--	3-F, 4-CF <sub>3</sub>		572	B
1046	Na	--	3,4-Cl		490	B
1047	Na	--	3,4-Cl		553	B
1048	Na	α-F	3,4-Cl		572	B
1049	Na	--	3,4-Cl		600*	B
1050	Na	--	3,4-Cl		490	B
1051	Na	α-F	3,4-Cl		572	B

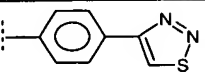
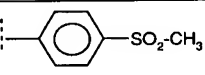
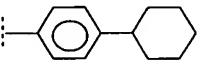
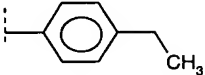
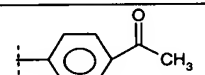
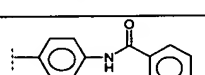
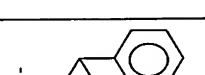
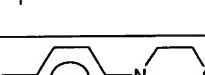
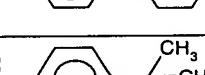
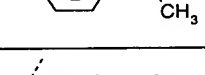
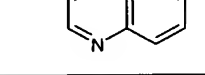
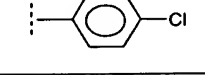
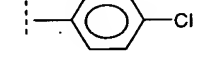
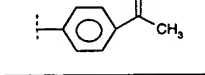
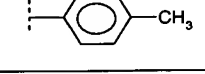
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1052	Na	--	3,4-Cl		594	C
1053	Na	--	3,4-Cl		588	B
1054	Na	--	3,4-Cl		592	C
1055	Na	--	3,4-Cl		538	B
1056	Na	--	3,4-CH <sub>3</sub>		512	B
1057	Na	--	3,4-Cl		629	B
1058	Na	--	3,4-Cl		550	B
1059	Na	--	3,4-F		563	B
1060	Na	--	3,4-Cl		566	B
1061	Na	--	3,4-F		529	A
1062	Na	--	3,4-F		512	A
1063	Na	--	3,4-Cl		546	B
1064	Na	--	3,4-F		520	A
1065	Na	--	3,4-Cl		524	B
1066	Na	--	3,4-Cl		550	C

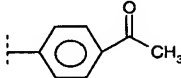
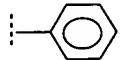
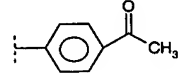

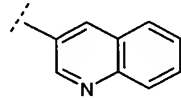
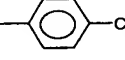
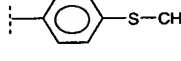
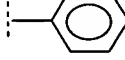
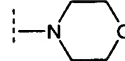
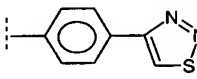
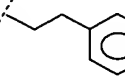
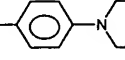
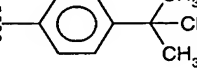
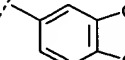
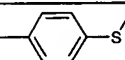
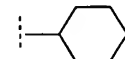
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1067	Na	--	3-F, 4- CF <sub>3</sub>		570	A
1068	Na	--	3,4-F		478	A
1069	Na	<i>b</i> -Br	3,4-Cl		629	A
1070	Na	--	3,4-Cl		534*	B
1071	Na	--	3,4-CH <sub>3</sub>		519*	A
1072	Na	--	3,4-Br		611	C
1073	Na	--	3,4-F		524	A
1074	Na	--	3,4-Br		599	C
1075	Na	--	3,4-Br		606*	B
1076	Na	--	3,4-Br		681	C
1077	Na	--	3,4-Cl		537*	C
1078	Na	--	3,4-Br		684	C
1079	Na	--	3,4-Br		673 (M+18)	A
1080	Na	--	3-CN		511	A
1081	Na	--	3,4-Br		646	C
1082	Na	--	3,4-Cl		516	B

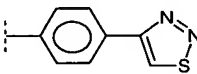
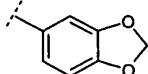
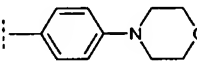
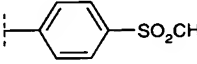
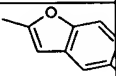
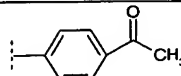

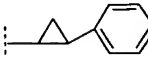

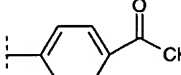
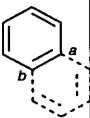
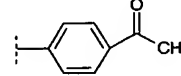
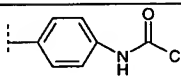
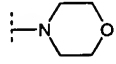
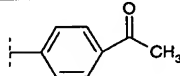

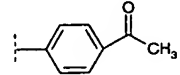
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1083	Na	--	3,4-F		562	B
1084	Na	--	3,4-Br		644	C
1085	Na	--	3-CN		552	A
1086	Na	--	3,4-Br		676	C
1087	Na	--			558	A
1088	Na	--	3,4-Br	 stereochemistry undetermined	639	B
1089	Na	--	3,4-Br	 stereochemistry undetermined	639	C
1090	Na		3,4-Cl		602	A
1091	Na		3,4-Cl		602	A
1092	Na	--	3,4-Br		654.8*	C
1093	Na	--	3-Cl, 4-F		503	A
1094	Na	--	3-Cl, 4-F		536	B
1095	Na		3,4-Cl		602	A

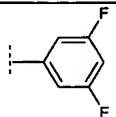
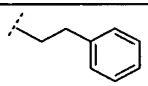
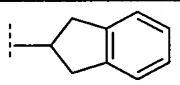
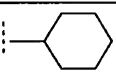
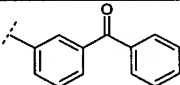
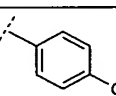
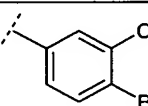
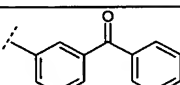
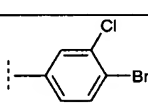
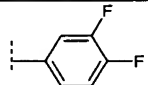
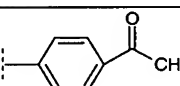
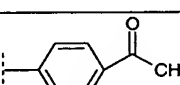
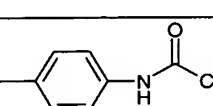
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1096	Na	--	3,4-Cl		546	B
1097	Na	--	3,4-Br		627.9	C
1098	Na	--	3,4-Cl		550	B
1099	Na	--	3,4-Br		606	C
1100	Na	--	3,4-Cl		614	B
1101	Na	--	3,4-Cl		544	B
1102	Na	--	3,4-Br		709	B
1103	Na	--	3,4-Br		701.9	B
1104	Na	--	3,4-Cl		623.9	B
1105	Na	--	3,4-Br		635.9	B
1106	Na	<i>b</i> -F	3,4-Cl		570	B
1107	Na	<i>c</i> -F	3,4-Cl		570	B
1108	Na	--	3,4-Cl		565*	B

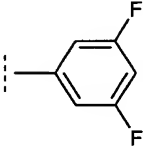

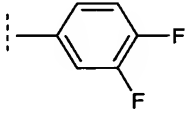
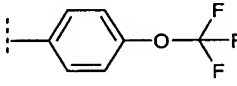
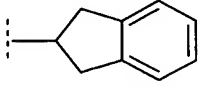
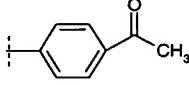
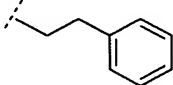
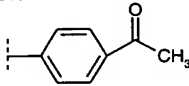
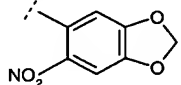
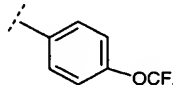
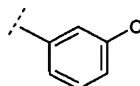
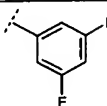
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1109	Na	--	3,4-Br		336	B
1110	Na	--	3,4-Br		634	C
1111	Na	--	3,4-Cl		546	B
1112	Na	--	3,4-Cl		594	B
1113	Na	--	3,4-Br		638*	C
1114	Na	<i>o</i> -Cl	3,4-Cl		586	B
1115	Na	--	3-Cl, 4-F		522	B
1116	Na	<i>b</i> -Cl	3,4-Cl		586	A
1117	Na	--	3,4-Cl		599	B
1118	Na	--	3,4-Br		684	B
1119	Na	--	3,4-Br		634	C
1120	Na	--	3-Cl, 4-F		530	B

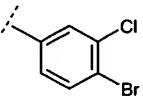
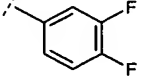
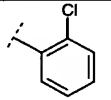
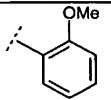
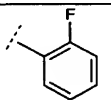
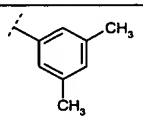
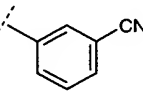
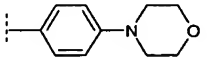
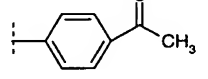
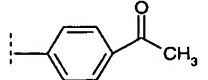
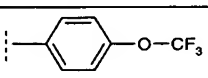
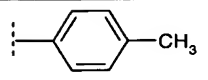
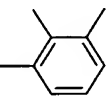
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1121	Na	--	3-Cl, 4-F		608	B
1122	Na	--	3-Cl, 4-F		530	A
1123	Na	--	3,4-Cl		544	C
1124	Na	--	3,4-Cl		540	B
1125	Na	--	3,4-Cl		528	C
1126	Na	--	3,4-Cl		538	B
1127	Na	--	3,4-Cl		535	B
1128	Na	--	3,4-Cl		590	B
1129	Na	<i>o</i> -OMe	3,4-Cl		581.9	A
1130	Na	<i>b</i> -OMe	3,4-Cl		579.9*	A
1131	Na	--	3-Cl, 4-F		578	B
1132	Na	--	3,4-F		492	A
1133	Na	--	3,4-Cl		536*	B

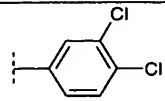
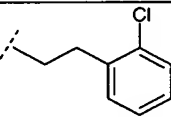
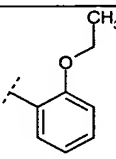
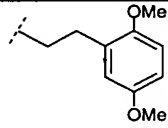
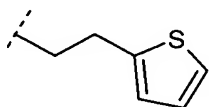
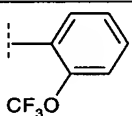
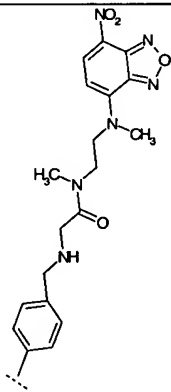
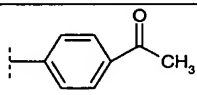
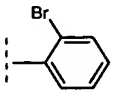
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1134	Na	--	3,4-Br		665.6*	C
1135	Na	--	3,4-Cl		570*	C
1136	Na	--	3,4-Cl		554	A
1137	Na	--	3,4-Cl		598	B
1138	Na	--	3,4-Cl		544	C
1139	Na	--	3,4-Cl		594	B
1140	Na	--	3,4-Cl		830	B
1141	Na	--	3- NHC(O) (CH <sub>2</sub> ) <sub>3</sub> C H <sub>3</sub> , 4-Cl		617	B
1142	Na	--	3,5-Cl		590	C

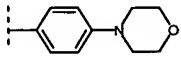
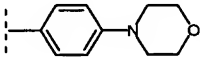
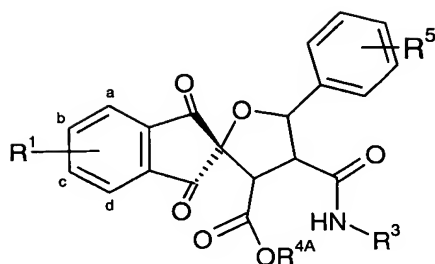
Table 1 cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
1143	Na	b-F	3,4-Br		702.9	C
1144	Na	c-F	3,4-Br		702.9	C

TABLE 1A



enantiomerically pure, "*cis/cis*" isomer (form 1)

Table 1A cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
A1001	Na	--	3,4-Br	 stereochemistry undetermined	683.8	A
A1002	Na	--	3,4-Br	 stereochemistry undetermined	683.8	C
A1003	Na	mixture <i>b</i> -Me & <i>c</i> -Me	3,4-Cl	 stereochemistry undetermined	568	A
A1004	Na	<i>b</i> -Me	3,4-Cl	 stereochemistry undetermined	568	A
A1005	Na	<i>c</i> -Me	3,4-Cl	 stereochemistry undetermined	568	C

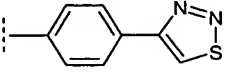
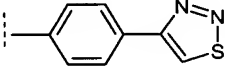
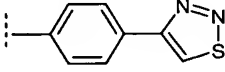
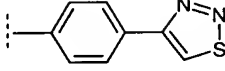
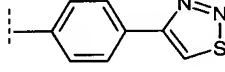
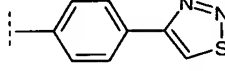
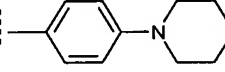
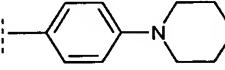
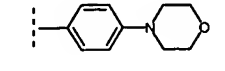
Table 1A cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
A1006	Na	mixture <i>b</i> -Me & <i>c</i> -Me	3,4-Cl	 stereochemistry undetermined	608	A
A1007	Na	<i>b</i> -Me	3,4-Cl	 stereochemistry undetermined	608	B
A1008	Na	<i>c</i> -Me	3,4-Cl	 stereochemistry undetermined	608	C
A1009	Na	mixture <i>b</i> -Me & <i>c</i> -Me	3,4-Br	 stereochemistry undetermined	697.9	A
A1010	Na	<i>b</i> -Me	3,4-Br	 stereochemistry undetermined	697.9	B
A1011	Na	<i>c</i> -Me	3,4-Br	 stereochemistry undetermined	697.9	C
A1012	Na	--	3,4-Br	 stereochemistry undetermined	683*	A
A1013	Na	--	3,4-Br	 stereochemistry undetermined	683*	C
A1014	Na	<i>c</i> -Me	3,4-Br		699	C

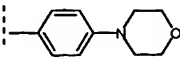
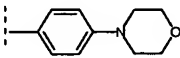
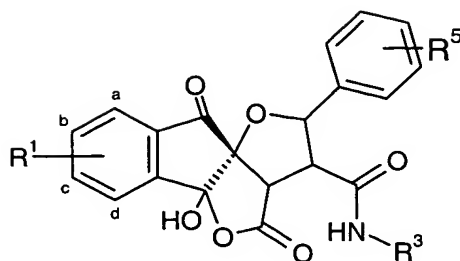
Table 1A cpd #	R <sup>4A</sup>	R <sup>1</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
A1015	Na	b-F, c-Me	3,4-Br		716.8	C
A1016	Na	b-Me, c-F	3,4-Br		717	B

TABLE 1B



enantiomerically pure, "*cis/cis*" isomer (form 3)

Table 1B cpd #	R <sup>1</sup>	R <sup>5</sup>	R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
B1001	b-Me, c-Me (mixture)	3,4-Br		699	A
B1002	b-Me	3,4-Br		699	C
B1003	c-Me	3,4-Br		699	C
B1004	b-Me	3,4-Br		676	A
B1005	c-Me	3,4-Br		676	A
B1006	b-Me	3,4-Br		676	B
B1007	c-Me	3,4-Br		676	C
B1008	b-F, c-Me	3,4-Br		716.6	C

TABLE 1C

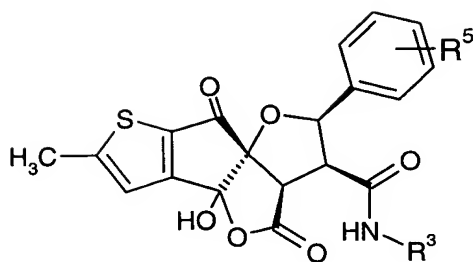
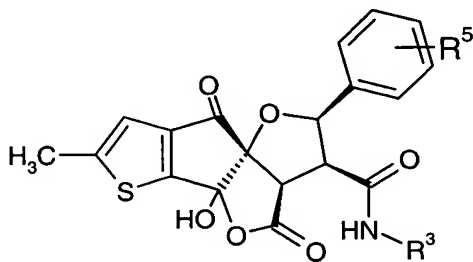


Table 1C cpd #	R <sup>5</sup>	R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
C1001	3,4-Cl		572	A
C1002	3,4-Br		704	B
C1003	3,4-Br		747	B

TABLE 1D

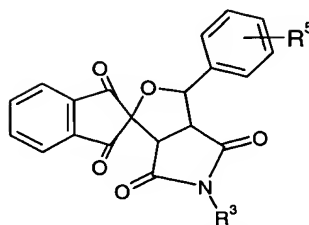


enantiomerically pure "*cis/cis*" isomer (form 3)

Table 1D cpd #	R <sup>5</sup>	R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
D1001	3,4-Cl		572	C
D1002	3,4-Br		704	C



TABLE 2



racemic, "cis/cis" isomer (form 2)

Table 2 cpd #	--R <sup>5</sup>	---R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
2001	4-Cl		500*	A
2002	4-Cl		516*	A
2003	4-Cl		498*	A
2004	4-Cl		474*	A
2005	3-Cl		500*	B
2006	4-Cl		470*	A
2007	4-Cl		490*	A
2008	4-CF <sub>3</sub>		534*	A
2009	4-Cl		490*	A
2010	4-Cl		470*	A
2011	4-Cl		524*	A

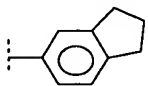
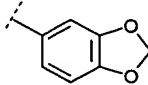
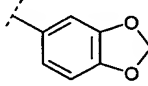
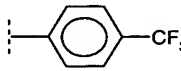
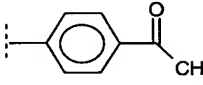
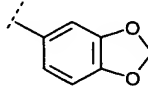
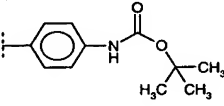
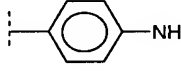
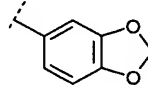
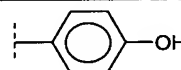
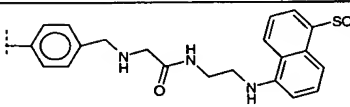
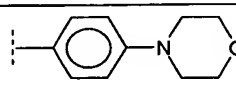
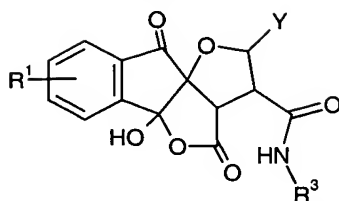
Table 2 cpd #	--R <sup>5</sup>	---R <sup>3</sup>	ES MS (M+H) <sup>+</sup> *(M-H)	IC <sub>50</sub> (μM)
2012	4-Cl		496*	A
2013	3,4-Cl		534*	B
2014	3-CH <sub>3</sub>		480*	A
2015	4-Cl		524*	A
2016	3,4-Cl		552 (M+18)	B
2017	4-I		612 (M+18)	A
2018	3,4-Cl		624 (M+18)	A
2019	3,4-Cl		524 (M+18)	B
2020	4-OH, 5-Cl		534 (M+18)	B
2021	3,4-Cl		526 (M+18)	C
2022	3,4-Cl		827	A
2023	3,4-Br		667	C

TABLE 3



racemic, "cis/cis" isomer (form 3)

Table 3 cpd #	R <sup>1</sup>	--Y	--R <sup>3</sup>	ES MS (M+H) + *(M-H)	IC <sub>50</sub> (μM)
3001	--			552*	B
3002	--			482	A
3003	--			488*	A
3004	--			566*	A
3005	--			518*	A
3006	--			510	A
3007	--			538	A
3008	--			637	A
3009	--			552	C

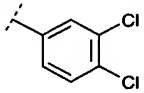
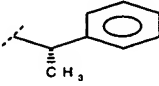
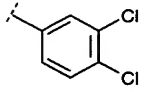
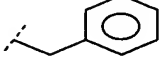
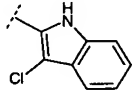
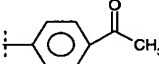
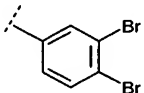
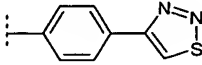
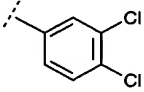
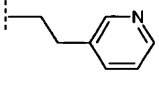
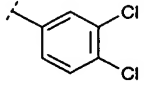
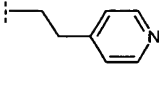
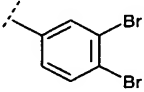
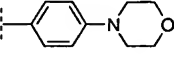
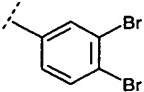
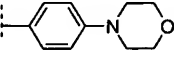
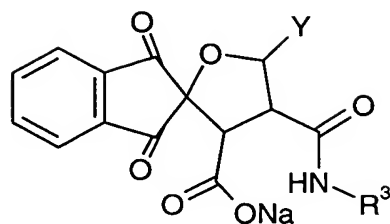
Table 3 cpd #	R <sup>1</sup>	--Y	--R <sup>3</sup>	ES MS (M+H) + *(M-H)	IC <sub>50</sub> (μM)
3100	--			540	A
3011	--			526	B
3012	--			569*	B
3013	c-Me			697	C
3014	--			588	B
3015	--			544	B
3016	b-F			702	C
3017	c-F			702	C

TABLE 4

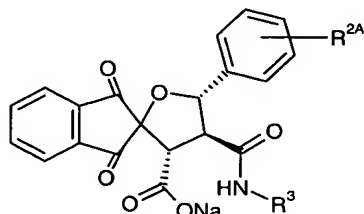


racemic, "cis/cis" isomer (form 1)

Table 4 cpd #	--Y	--R <sup>3</sup>	ES MS (M+H) + *(M-H)	IC <sub>50</sub> (μM)
4001			536	B
4002			526	A
4003			530	A
4004			534	B
4005			510	A
4006			588	A
4007			588	B
4008			523	A
4009			523	A

Table 4 cpd #	--Y	--R <sup>3</sup>	ES MS (M+H) + *(M-H)	IC <sub>50</sub> (μM)
4010			576*	B
4011			522*	A
4012			589	A

TABLE 5

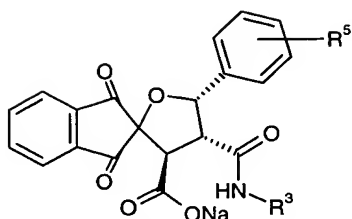


racemic, *trans/trans* isomer<sup>†</sup> (form 1)

Table 5 cpd #	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H)	IC <sub>50</sub> (μM)
5001	3,4-Cl		554	B

<sup>†</sup> relative stereochemistry shown

TABLE 6

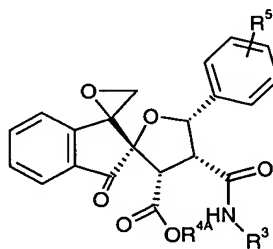


racemic, *trans/cis* isomer<sup>‡</sup> (form 1)

Table 6 cpd #	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup>	IC <sub>50</sub> (μM)
6001	3,4-Cl		568	A

<sup>‡</sup> relative stereochemistry shown

TABLE 7

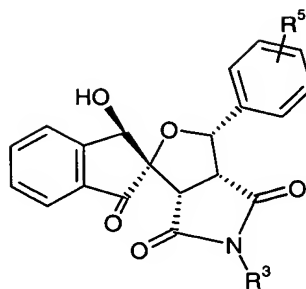


racemic, "*cis/cis*" isomer<sup>‡</sup> (form 1)

Table 7 cpd #	R <sup>4A</sup>	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup>	IC <sub>50</sub> (μM)
7001	OCH <sub>3</sub>	3,4-Cl		582	A

<sup>‡</sup> relative stereochemistry shown

TABLE 8

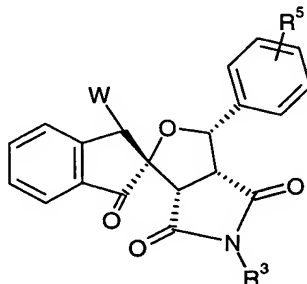


racemic, "cis/cis" isomer<sup>†</sup> (form 2)

Table 8 cpd #	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup>	IC <sub>50</sub> (μM)
8001	3,4-Cl		556	B

<sup>†</sup> relative stereochemistry shown

TABLE 9

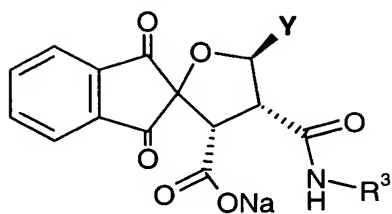


racemic, "cis/cis" isomer<sup>†</sup> (form 2)

Table 9 cpd #	W	--R <sup>5</sup>	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup>	IC <sub>50</sub> (μM)
9001		3,4-Cl		540	A

<sup>†</sup> relative stereochemistry shown

TABLE 10



racemic, *cis/trans* isomer<sup>‡</sup> (form 1)

Table 10 Cpd #	--Y	--R <sup>3</sup>	ES MS (M+H) <sup>+</sup>	IC <sub>50</sub> (μM)
10,001			683	B

<sup>‡</sup> relative stereochemistry shown